INTERACTIONS BETWEEN NEMATODE AND FUNGAL PATHOGENS OF THE CITRUS FIBROUS ROOT CORTEX

By

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To The Memory Of My Brother Samir
My Mother and My family

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INTERACTIONS BETWEEN NEMATODE AND FUNGAL PATHOGENS OF THE CITRUS FIBROUS ROOT CORTEX

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The most commonly encountered association between nematodes and fungi in citrus occurs between the citrus nematode *Tylenchulus semipentrans* Cobb and the root rot fungus *Phytophthora nicotianae*(=*parasitica*) Dastur Breda de Hann. The fibrous root system of citrus trees in Florida and worldwide is commonly infected by both parasites. The efficacy of nematicides to manage *T. semipenetrans* in field experiments was directly related to the population density of *P. nicotianae*, implying significant competition between these two parasites. We initiated a study of this hypothesis by testing whether infection of root segments by the citrus nematode impeded root infection by *P. nicotianae* in vitro, and by determining the effect of the nematode on growth and pathogenicity of the fungus in whole plant experiments. Plants were infected by the

fungus, the nematode, both organisms or neither organism. Infection of roots by *T. semipenetrans* reduced subsequent infection by *P. nicotianae* and damage to the plant. Citrus seedlings infected by both organisms grew larger and contained less fungal protein in the root tissues than did plants infected by only the fungus thus demonstrating antagonism of the nematode to the fungus.

Two hypotheses were investigated to explain the mechanism of the interaction between these pathogens:1) indirect mediation through increased colonization of nematode feeding sites by microorganisms antagonistic to *P. nicotianae,* and 2) direct antibiosis by the nematode. To test the first hypothesis, a field survey was initiated to determine whether infection by *T. semipenetrans* changes the composition of rhizosphere inhabiting microorganisms, and to identify microorganisms that are consistently associated with the nematode. Results showed that *T. semipenetrans* altered the microbial community in the citrus rhizosphere by increasing propagule densities of bacteria and fungi. The dominant bacterial species isolated were *Bacillus megaterium* and *Burkholderia cepacia.* Both bacteria were used in whole plant factorial experiments with *P. nicotianae* and *T. semipenetrans* either alone or in combination. Neither bacterium inhibited growth of the fungus when inoculated alone. Nevertheless, the nematode and both bacteria increased the growth of citrus seedlings infected by *P. nicotianae.*

To test the second hypothesis, *in vitro* bioassays were conducted to determine the effects of eggs of two nematodes *T. semipenetrans* and *Meloidogyne arenaria* on mycelial growth of *P. nicotianae* and *Fusarium solani. Tylenchulus semipenetrans* eggs suppressed mycelial growth of *P. nicotianae* and *F. solani in vitro,* but *M. arenaria* eggs

had no comparable effect on either fungus. This research showed an antagonistic effect of a plant parasitic nematode on a plant pathogenic fungus and shows potential mechanisms involving direct inhibition of the fungus by the nematode and indirect mitigation of fungal virulence mediated by complex microbial interactions in the citrus rhizosphere.

CHAPTER 1
GENERAL INTRODUCTION

Citrus is the most economically important crop in Florida with revenues exceeding one billion dollars every year. Approximately 336,817 ha of citrus are grown in Florida. Of these, 80% are orange, 14% are grapefruit, and 6% are specialty fruit (Florida Agriculture Statistics Service 2000). The most important citrus-growing regions in Florida are located in Polk, Hendry, Highlands, DeSoto, Hardee, St. Lucie, Indian River, and Martin counties. These eight counties comprised about 75% of the total area of citrus production in Florida (Florida Agriculture Statistics Service 2000).

More than 100 biotic and abiotic factors cause diseases of citrus trees (Whiteside et al. 1988). Two of the most important biotic factors for young and mature citrus trees are fungi and nematodes. Diseases caused by soilborne fungi, such as *Phytophthora* spp. result in root rot; foot rot; brown rot of fruit; reduced fruit quality and yield; and under optimum conditions for disease trees may be killed (Timmer et al. 1989). Citrus nematode *Tylenchulus semipenetrans* (Cobb 1914) is ubiquitous in commercial citrus growing regions worldwide. The nematode is the causal agent of the disease citrus slow decline. The fibrous root systems of citrus trees are commonly infected by both *T. semipenetrans* and *Phytophthora nicotianae* Dastur Breda de Haan (synonym = *P. parasitica*) (Hall 1993). Both organisms feed in the cortex of fibrous roots and both have been shown to reduce the density of the fibrous root system (Duncan et al. 1993; Graham and Menge 1999). In a study of the effects of managing concomitant populations of

Phytophthora nicotianae and *Tylenchulus semipenetrans,* Graham and Duncan (1997) showed that yields responded in a density-dependent manner to the control of either or both parasites. However, management of both pathogens did not substantially increase yields more than management of either alone. Reduction of either population increased the population density of the other. Increased root growth after fungus control is responsible for increased nematodes, because the numbers of nematodes per mass of root did not increase. Root mass did not increase following nematicide treatments, whereas there was a highly significant inverse relationship between numbers of nematodes and numbers of propagules of *P. nicotianae* in the soil. This suggests that either the nematode or an associated agent was producing antibiotics affecting the fungus, or that the nematode competed for resources with the fungus. These results were supported by a greenhouse study in which *T. semipenetrans* interfered with *P. nicotianae,* reducing levels of infection in roots and producing increased growth of citrus seedlings compared with seedlings infected by *P. nicotianae* alone. The research revealed a significant interaction between the nematode and the fungus that may be of economic importance. The objectives of this research were to extend the findings of Graham and Duncan (1997) by:

- determining the nature of the association (additive, or antagonistic or synergistic) between citrus nematode *Tylenchulus semipenetrans* and root rot fungus *Phytophthora nicotianae* on citrus plants.
- determining the mechanism(s) underlying the interaction.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

Under natural conditions a plant is a potential host to various microorganisms that can influence each other, particularly when they occupy the same niche. It is reasonable to expect that infection by one pathogen may alter the host physiology and thereby subsequent infection by another organism (Taylor, 1990). Pathogens also affect one another through resource competition for nutrients (Elad and Chet, 1987, Suslow, 1982, Weller 1985), competition for infection sites, (Baker, 1968, Osburn et al., 1989) or parasitism and production of lytic enzymes (Mitchell and Alexander, 1961; Mitchell and Hurwitz, 1965; Sneh, 1981). Weller (1988) reported that nutrients, rather than space, are thought to be the limiting factor in competition among rhizosphere microorganisms during colonization. Plant-parasitic nematodes often play a major role in disease interactions involving a variety of other organisms. Interactions involving nematodes are important because they contribute substantially to variability in crop growth (Zadoks and Schein, 1979). The most commonly cited interactions involving nematodes include the following:

- Synergistic disease complexes (Powell, 1971a, 1971b, 1979; Golden and Van Gundy, 1975).
- Antagonism, or disease inhibition (Jorgenson, 1970, Gray et al., 1990).
- Reduced resistance to subsequent infection (Harrison and Young, 1941; Webster,

1985; Hasan, 1985; Khan and Nejad-Hosseini, 1991).

- Increased resistance against a subsequent parasite (Sidhu and Webster, 1981).

Defining Interaction

The term interaction is used widely in plant nematology for various kinds of associations and its precise usage is rather rare. Both quantitative and qualitative responses resulting from two or more factors involved in plant diseases have been described as interaction in nematology literature. Nematodes like all organisms come into association with other organisms in the course of their existence. Without any consideration whether or not "interaction" (in the mathematical sense) operates, biologists are prone to see "interaction" when terms like "association," "relationship" or just "relation" suffice (Burrows, 1987). Wallace (1983) suggested that use of the term "interaction" should be restricted to quantitative plant disease interactions showing synergism or antagonism. This would be interaction in the statistical sense rather than in the descriptive sense. According to Burrows (1987), all nonstatistical qualitative interactions can be simply termed "relationship" or "relation."

Similarly, use of the term "synergism" and "antagonism" has been variable, with different connotations. Dickinson (1979) described how the word synergism was introduced in plant pathology and was defined as "an association of two more organisms acting at one time and affecting a change that only one is not able to make." Powell (1979) defined synergism as "the concurrent or sequential pathogenesis of host plant by two or more pathogens in which the combined effects of the two pathogens are greater than the sum of the effects of each pathogen alone." The first definition is somewhat more qualitative whereas the definition of Powell (1979) is quantitative and more useful in nematology. In statistics, the term synergism is used to indicate a positive interaction (

i.e. the sum of the treatment effects is not simply additive) (Wallace, 1983, 1989). Wallace (1983) proposed the avoidance of the terms synergism and antagonism altogether and suggested that the events could be adequately described by the terms positive and negative interactions, respectively.

The statistical definitions of additivity and interaction (antagonistic or synergistic) are used in this dissertation. It should be noted, however, that the statistical definition of additivity is somewhat at variance with normal usage of the word in a biological sense (Duncan and Ferris, 1982). These differences are shown if we consider the plant or nematode response to increasing population densities of a single nematode species. A typical damage function describing yield as a function of initial nematode population density (Pi) is sigmoid. The relationship can be linearized by log transformation of Pi. Thus, each successive increment of yield loss is associated with increasingly larger cohorts of pathogens. These functions illustrate that each successive infection by a nematode causes proportionately less yield loss than did the preceding nematodes, and they represent an additive effect. The same phenomenon occurs with respect to population growth. Because of intraspecific competition for resources, population growth rate is inversely proportionate to Pi. The biological and mathematical basis for this phenomenon was described by Nicholson (1933) and extended to nematology by Seinhorst (1965). Seinhorst's model was extended to describe more than a single nematode species (Duncan and Ferris, 1982). In practical terms, additive effects on yield or population growth involving one or several species can be predicted by these models. Therefore, any system involving more plant damage or population growth than predicted by these models could be considered synergistic, while any involving less damage or

population growth could be considered antagonistic. Any system predicted by the models would be additive; that is, no interaction occurred.

Tylenchulus semipenetrans

Tylenchulus semipenetrans was observed for the first time in 1912 by J. R. Hodges, a horticultural inspector for Los Angeles County, California (Thomas, 1913). Subsequent reports showed that this nematode is widespread and occurs in all citrus-producing regions of the world (Heald and O'Bannon, 1987; Van Gundy and Meagher, 1977). The citrus nematode is the causal agent of a disease called citrus slow decline. This disease is so named because the nematode population increases slowly throughout the root system and requires several years to debilitate trees and reduce fruit yield (Cohn et al., 1965; Reynolds and O'Bannon, 1963). Symptoms of the disease appear sooner when trees are replanted in infested soil. High population densities of *T. semipenetrans* in replanted soil was reported to kill young trees within the first year of replanting (Thorne, 1961).

Biology of *Tylenchulus semipenetrans*

The life stages of *T. semipenetrans* consist of the egg stage, four juvenile stages (J1-J4) and the adult stage (Cobb, 1914; Van Gundy, 1958). The life cycle from egg to egg is completed within 6-8 weeks, depending on the host, average soil temperature and other environmental factors (Cohn, 1965; O'Bannon et al., 1966; Van Gundy, 1958). Development of the first-stage juvenile proceeds within the egg where the first molt occurs (Gutierrez, 1947). All stages parasitize root parts except eggs, J1 and males (Van Gundy, 1958). The J2, J3, and J4 stages feed on young citrus feeder roots (epidermal and hypodermal cells (Cohn, 1965; Schneider and Baines, 1964; Van Gundy and Kirkpatrick, 1964). Juveniles take approximately 2 weeks to become associated with the roots and

begin feeding on the epidermal cells (Van Gundy, 1958). Penetration occurs 19 days after inoculation on average. Then immature females (J4) complete the infection phase within 5 to 6 days (Cohn, 1964) by penetrating deeply into feeder root cortical tissue. The females increase in size becoming posteriorly swollen and saccate. The mature female is a sessile semi-endoparasite. The anterior portion of the female extends several cell layers deep in the cortical parenchyma, whereas the posterior portion of the body enlarges outside the root. The nematode begins to lay eggs, that remain attached to the body in a gelatinous matrix on the root surface (Van Gundy, 1958). The eggs and the gelatinous matrix together with its contents are known as eggmasses (Maggenti, 1962). Egg masses contain up to 75 to 100 eggs (Baines, 1950). Reproduction is facultatively parthenogenic and the mature female lays about 500 eggs during her lifetime (Van Gundy, 1958).

Females feed in the fibrous root cortex where they became immobile establishing permanent specialized feeding sites. The feeding site consists of 6 to 10 "nurse" cells around the nematode head (Van Gundy, 1958). The "nurse" cells are required for successful reproduction and die when the female dies. Nurse cell histology shows the cytoplasm to be dense, but devoid of starch, presumably a primary nutrient of the nematode (Cohn, 1965; Duncan et al., 1994). Root penetration may extend to the endodermis (Cohn, 1964; Van Gundy, 1958) and damage is usually pronounced in the cortex. Heavily infected roots show extensive necrosis which gives them an abnormally dark color compared to noninfected roots. The cortex of the affected region sloughs off, resulting in a shortened, irregular appearance or death of the affected rootlet (Cohn, 1965; Reynolds and O'Bannon, 1963).

Damage Symptoms

Symptoms of slow decline disease vary and are associated with root disfunction. Reduced root terminal growth was the first reported clinical symptom (Thomas, 1913). At high population density (4,000 females/gram of root), trees generally exhibit low vigor, chlorosis, canopy thinning, twig dieback, smaller than normal fruit and reduced yield (Cohn et al., 1965). Symptoms of slow decline vary with soil environment. In California high population densities of nematodes are invariably associated with reduced fruit yield but not always associated with decline symptoms in the tree (Baines et al., 1978). Population densities of the nematodes in Florida and other parts of the humid tropics and subtropics are generally lower than those from dryland or Mediterranean climates (Duncan and El Morshedy, 1996). O'Bannon (1968) found that in well-drained deep sandy soils in the central ridge of Florida, the population densities of *T. semipenetrans* on mature trees may exceed 5,000 juveniles/g fresh roots, without showing distinct decline or visual symptoms. On the other hand, population densities of *T. semipenetrans* below 1000 juveniles/g roots were related to severe decline and more obvious symptoms in the poorly drained soil in the coastal areas of Florida where organic matter is high; soil is more shallow and the water table and salinity are higher.

Environmental Factors

Many biotic and abiotic factors have been shown to influence population densities of *T. semipenetrans*. Gutierrez (1947) was the first to report that temperature greatly influenced development of the citrus nematode *T. semipenetrans*. Only slight nematode infection of orange roots occurred at 15 or 35 °C. The optimum nematode infection and development was observed between 25 and 30 °C (Baines, 1950). Van Gundy (1958)

showed that at 25 °C the life cycle from egg to egg required 6-8 weeks. O'Bannon et al. (1966) showed that the optimum average temperature for *T. semipenetrans* population development is 25 °C and the percentage of infection was reduced and reproduction was delayed at 30 °C. At 25 °C, 80% of penetration occurred when the roots were in the primary stage of development and before the appearance of secondary xylem (Cohn, 1964). Van Gundy (1984) showed that the juveniles are not active when mean soil temperature is below 16 °C. Van Gundy and Tsao (1963) reported that *T. semipenetrans* reproduction begins at 21 to 22 °C, reached a maximum between 28 and 31 °C and ceased at 31 °C.

In Florida *T. semipenetrans* females have the highest rate of development in summer through autumn (July-Nov.) but development declines during winter (Dec.-March) and reaches the lowest levels during midsummer (O'Bannon et al., 1972; Duncan and Cohn, 1990). O'Bannon (1968) showed that fall populations are generally higher than spring populations because the reduction in nematode numbers is not as great during the summer months. From January to March and July to September conditions are not favorable for citrus nematode activity (O'Bannon, 1968).

Van Gundy and Martin (1961) found higher *T. semipenetrans* population densities in alkaline than in acid soils. The optimum soil pH for *T. semipenetrans* development is 6.0-7.5 (Van Gundy et al., 1964), but infection occurs at low soil pH (Bello et al., 1986; Davide, 1971; Martin and Van Gundy, 1963; Reynolds et al., 1970).

Soil with a high organic matter content can greatly influence and favor the initial rate of nematode infection and subsequent reproduction. Van Gundy et al. (1964) found that nematode infected citrus seedlings grown in soil containing 10-15% clay had the

highest rate of nematode reproduction and the greatest plant growth reduction. Van Gundy (1958) and O'Bannon et al. (1966) suggested that peat moss created a thin protective cover layer over infected citrus roots that enhanced nematode infectivity and that *T. semipenetrans* females accumulate in the greatest numbers by adding peat moss to soil.

The production and development of eggs of citrus nematode were reduced in soils with low oxygen availability (Stolzy et al., 1963). Population development of *T. semipenetrans* was favored by dry rather than wet soil (Van Gundy et al., 1964). The reduction of soil oxygen due to excess soil moisture generally reduces *T. semipenetrans* population densities (Van Gundy et al., 1964; Norton, 1978). Ayoub (1980) found that the population densities of *T. semipenetrans* increased when heavy rains were interrupted by short drought spells suggesting that juveniles and eggs were possibly washed out of the gelatinous matrix by rain. Soil moisture has a major effect on the seasonal variation in population density of *T. semipenetrans* in sandy soil in Florida citrus orchards compared to other climatic factors. Duncan et al. (1993) showed that seasonal patterns of root quality and annual differences in root abundance and quality were related to populations of both the *T. semipenetrans* and *P. nicotianae*. Patterns of change in root mass density and concentration of root lignin and nonstructural carbohydrate suggested annual as well as seasonal variation in the age structure and nutritional value of the fibrous root system. Numbers of nematodes were related inversely ($P \leq 0.01$) to soil moisture and root lignin content, and positively related to starch concentration. The population density of *T. semipenetrans* tends to be higher in drier climates (Cohn, 1966) than in humid tropics and subtropics (Duncan, et al. 1993, O'Bannon, et al. 1972). In

drier climates (Mediterranean and Florida central ridge) where root systems are unrestricted by high water tables, citrus produces a deep root system that maintains high levels of *T. semipenetrans* when water is available from other portions of the root system. In humid tropics and subtropics (Florida coastal area) where root systems are restricted due to high water tables, the density of *T. semipenetrans* may be regulated by high soil moisture during the rainy season and by complete drought during the dry season. The major factor in the population differences in these regions is suggested to be moisture availability in surface soils (Duncan and El-Morshedy, 1996). In a greenhouse study (Duncan and El-Morshedy, 1996) in which citrus seedlings were grown in vertical tubes with upper and lower sections, the population development of *T. semipenetrans* responded differently to dry sandy soils depending on whether all or part of the root system experienced drought. Under local drought (only lower section was irrigated), number of eggs, juveniles and males in the soils and per gram of roots were higher than those under nondrought or uniform drought (both upper and lower section were irrigated or neither sections was irrigated) respectively. There was no survival for the nematodes under uniform drought in soil. They also suggested that the hydraulic lift through the root xylem may prolong the activity of the nematodes in dry soils and other rhizosphere organisms.

The interaction between salinity and *T. semipenetrans* has a major impact on citrus. More citrus nematodes and other nematodes were also found around citrus roots subjected to continuous high, though tolerable salinity, than around citrus roots grown at lower salinity levels (Machmer, 1958). High *T. semipenetrans* population densities were recovered from mature trees irrigated with various soluble salts compared with lower

population densities from trees irrigated with surface water. Cohn (1976) reported that in South Africa, the highest population densities of *T. semipenetrans* (10,000-40,000 juveniles/g fresh root) are commonly associated with saline conditions whereas the lowest population densities (100-500 juveniles/g fresh root) were associated with nonsaline conditions. In Israel, the highest population densities of *T. semipenetrans* occur in the more saline coastal or desert regions (Cohn et al., 1965; Heller et al., 1973). Mashela et al. (1992) studied the interaction between *T. semipenetrans* and salinity showing that salinity increases population densities of *T. semipenetrans.* These salinity effects were most likely systemic because when nematodes and salinity were separated in seedlings with splitroots, nematode densities were higher than when nematodes were alone. Dunn et al. (1998) studied this relationship and showed *de novo* arginine biosynthesis as a response of citrus to salinity stress, concomitant with enhanced susceptibility to attack by *T. semipenetrans.* The effects of salinity stress on nematode behavior, arginine content and phenylalanine ammonia lyase (PAL) activity (PAL is a key phenolic chemical defense pathway enzyme) suggested that under stress, citrus grows more slowly and produces arginine in response to high levels of *in vivo* ammonia, resulting in lower PAL activity and decreased chemical defense against the nematode.

Phytophthora nicotianae

Phytophthora spp. are the causal agents of many devastating diseases, the most notable resulting in the famous Irish potato famine in Ireland 1845, caused by *P. infestans* (Klinkowski, 1970). *Phytophthora* spp. attack more than 2000 plant species worldwide, including *Citrus* spp. and other members of the Rutaceae family (Timmer and Menge, 1988). The most widespread and important *Phytophthora* spp. that attack citrus are *P.*

nicotianae and *P. citrophthora*. *Phytophthora nicotianae* Dastur Breda de Haan (synonym = *P. parasitica*) (Hall, 1993) is common and widespread in most citrus growing areas worldwide and causes foot rot, gummosis and root rot (Graham, 1990). Fibrous root rot is a common problem in citrus nurseries (Sandler et al., 1989) and about 90% of field nurseries in Florida are infested with *P. nicotianae* (Fisher, 1993). *Pytophthora nicotianae* does not live freely in the soil (Tsao, 1969) but obtains its nutrients from plant tissue (Lutz and Menge, 1986).

Biology of *Phytophthora* spp.

Phytophthora nicotianae can survive in soil or root debris as chlamydospores or oospores (Tsao, 1969). Under unfavorable conditions (cool temperature, soil poorly aerated, elevated carbon dioxide) chlamydospores are produced (Tsao, 1971) and can survive and persist in adverse conditions for a long period of time (Lutz and Menge, 1986; Malajczuk, 1983). When favorable conditions return, chlamydospores germinate directly to produce mycelia or indirectly to produce sporangia and zoospores (Mircetich and Zentmeyer, 1970).

Oospores occur when the A1 and A2 matings of the fungus are present. Oospores are produced in lower numbers than chlamydospores. Oospores have thick walls and are resistant to drying and cold temperature (Lutz and Menge, 1986). They require longer to mature than chlamydospores (Ribeiro, 1983) and remain dormant for extended periods of time (Malajczuk, 1983).

Sporangia are the primary reproductive structures and form during normal conditions of oxygen and carbon dioxide (Mitchell and Zentmeyer, 1971). Well-aerated moist soil is optimal for both production and germination of sporangia (Sommers et al., 1970). Sporangia may either germinate and form mycelium or release motile zoospores

(MacDonald and Duniway, 1978). Each sporangium releases from 5 to 40 zoospores, which can swim or be carried by moving water or roots. Zoospores are probably attracted to the zone of elongation of new roots by nutrients that are naturally excreted from this root zone (Morris and Ward, 1992). Once zoospores are in contact with the root, they encyst usually in the presence of high concentrations of amino acids (Khew and Zentmeyer, 1973). Zoospores can move over long distances between trees by water movement from rainfall or irrigation.

Damage Symptoms

Fibrous root rot disease is characterized by decay in the fibrous root cortex. The cortex becomes discolored and appears water soaked and then turns soft. The fibrous roots slough their cortex, leaving only the vascular tissue (the white, thread-like stele) (Whiteside et al., 1988). Root rot is more severe in susceptible rootstocks in infested nursery soil (Graham, 1995). Young fibrous roots of citrus are much more susceptible to root rot than older roots. The young trees may die due to the loss of a significant numbers of roots. In older trees, the trees decline, show low vigor, have smaller fruit size than normal, and have reduced yields (Graham and Timmer, 1992).

Environmental Factors

The minimum temperature for *Phytophthora* spp. growth varies from 5 to 7 °C with different isolates. The optimum temperature for *P. nicotianae* is 27 to 32 °C and the maximum is 37 °C. Hall (1993) reported that most isolates stopped growth at 5 and 35 °C and about half of the cultures were killed at 40 °C. Duncan et al. (1993) showed that in subtropical climates such as in south Florida, seasonal fluctuations in the population density are not consistent, although an overwinter decline does occur.

Numbers of fungal propagules in the soil and the amount of fungal protein in roots (as measured by ELISA) were directly related to concentration of ketone sugars in roots and to soil temperature and H_2O. *Phytophthora nicotianae* is most active during the warmer season. Chlamydospores and oospores are formed for survival during unfavorable periods. The release of zoospores is optimal in saturated soils (Mircetich and Zentmeyer, 1970). Root exudates released by living roots attract zoospores (Morris and Ward, 1992). Host susceptibility is increased when roots are stressed or damaged (Graham and Menge, 1999). Optimum condition for infection growth of *P. nicotianae* are present in soils with drainage restricted by hardpans or clay layers or those with shallow water tables that rise into the root zone (Graham and Menge, 1999). Frequency and duration of irrigation influence the activity of *P. nicotianae* and the predisposition of roots to rot. Excessive soil moisture can result in prolonged oxygen deprivation and makes roots more attractive to zoospores, increasing infection. In cool weather, populations of *P. nicotianae* decline in Mediterranean climates when the temperature is below 59°F, but in Florida the populations do not drop significantly because soil temperature rarely drops below 59°F (Graham and Menge, 1999).

Disease Complexes Involving Nematodes and Fungi

Various aspects of nematode-fungal interactions are well reviewed (Powell, 1971a; Bergeson, 1972; Webster, 1985; Mai & Abawi, 1987; Rowe et al., 1987; Evans & Haydock, 1993; France & Wheeler, 1993). The best known of these interactions occur between root-knot nematodes and Fusarium wilt fungi; however, root knot nematodes have long been known for their ability to predispose plants to infection by other secondary pathogens. The first published account of biopredisposition was by Atknison

(1892), who noted a very close relationship between *Meloidogyne* sp. and *Fusarium* wilt fungi in cotton. The combinations between these pathogens always contribute to synergistic interactions and more severe losses from wilt. Many studies have shown interactions among all major groups of parasitic fungi and a diversity of nematodes. In most interactions involving nematodes, the nematode plays the primary role modifying the host (Pitcher, 1978; Powell, 1979; Patel et al., 2000). With some exceptions, nematode infections make the host more susceptible or suitable to other pathogens. Some fungal pathogens have also been shown to render the host susceptible to the nematode (Hasan, 1985). Examples of synergism, antagonism or additive effects between nematode and fungi are described below.

Sedentary Endoparasitic Nematode: Nematodes enter plant tissues completely (sessile entirely within roots) or with a large portion of their body (sessile partly within roots). Root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp.) are often primary pathogens with the ability to predispose plants to heavier infection by other pathogens such as *Fusarium* spp., *Phytophothora* spp. and *Rhizocotonia* spp. Predisposing effects of *Meloidogyne* spp. were also noted in parasitism by secondary fungal pathogens in tobacco, such as *Curvularia*, *Botrytis*, *Penicilium*, and *Aspergillis*, that do not infect plants in the absence of predisposing factors (Powel et al., 1971b). In addition to those well-known associations, various systems involving root-knot, cyst and reniform nematodes show the three types of associations/interactions that can occur between fungi and nematodes.

Synergism: Disease complexes in which *Heterodera* spp are primary pathogens are well documented. Other pathogens involved include fungi in the genera *Fusarium,*

Phytophthora, Pythium, and *Rhizoctonia* (Powell, 1971a). Whitney (1974) showed that the effect on damping off of sugarbeet of *Pythium ultimum* and *Heterodera schachtii* in combination was synergistic. Roy et al. (1989) found similar synergistic effects in pathogenicity tests of two isolates of Fusarium from soybean plants with symptoms of sudden death syndrome (SDS). McLean and Lawrence (1993a) confirmed those findings and, using a split root system, showing that the influence of the nematode on SDS is localized rather than systemic. Rai and Singh (1996) reported that the wilt incidence and reduction in plant growth of pigeonpea increased when plants were inoculated with *Heterodera cajani* and *F. udum* in combination. The histopathological studies revealed that the main site of entry of *F. udum* is through the injury created by protruding females of *H. cajani.*

Walker et al. (1999) studied the relationship between root-knot nematode *Meloidogyne incognita* and the fungus *Thielaviopsis basicola* on cotton. Extensive vascular necrosis and sporulation within vascular tissue was observed in plants infected by both pathogens compared to plants grown in soil infested with *T. basicola* alone, which showed no evidence of vascular colonization by the fungus. They concluded that *M. incognita* greatly increases the access of *T. basicola* to vascular tissue. Jonathan and Rajendran (1998) found a synergistic interaction between *M. incognita* and *F. oxysporum* on banana (cv. Rasthali) both in concomitant and sequential inoculations, resulting in significant reduction in plant growth. The Panama wilt disease, in terms of corm rot, was significantly higher when nematode inoculation followed the fungus and in concomitant inoculations of the pathogens.

Synergistic interactions also occured between *M. javanica* and *F. oxysporum* f.sp.

ciceris on different chickpea cultivars both in concomitant and sequential inoculations where resistance of Pusa-212 to the fungus alone is broken in the presence of the nematode (Khan and Hosseini, 1991).

Predisposition of roots by *Meloidogyne* spp. for damage by different fungi have been reported on many crops. On tomato, inoculation of *M. incognita* 3 weeks before *R. solani* significantly reduced the tomato shoot weight and length compared with inoculation of *R. solani* 3 weeks before *M. incoginta* (Ferraz and Lear 1976; Singh et al., 1981). *M. incognita* and *Fusarium oxysporum* f. sp. *lycopersici*, acting alone caused characteristic root galling and shoot wilting, respectively, and significantly reduced plant growth and yield. In concomitant inoculation, severity of fusarial wilt was significantly increased and plant growth and yield reductions were also considerably greater compared to the sum of individual effects of the pathogens (Khan and Akram, 2000). Similar results have been shown between *M. incognita* and *F. oxysporum* f. sp. *coffeae* on coffee (Negron and Acosta (1989). On alfalfa, *M. hapla* increased the susceptibility of alfalfa to *Phytophthora megasperma* f. sp. *medicaginis* and increased the aggressiveness of the fungus and increased Phytophthora root rot and resultant plant mortality (Griffin et al., 1993; Griffin and Gray, 1994).

Antagonism: Gray et al. (1990) found that inoculated alfalfa seedlings were smaller after a single inoculation with only *Phytophthora megasperma* f. sp *medicaginis* than after inoculation with both *Meloidogyne hapla* and *P. megasperma* f. sp. *medicaginis*. Their explanation for the disease suppression is that the nematodes feeding on immature roots may have interfered with infection by *P. medicaginis*. Valle-Lamboy and Ayala, (1980) reported an antagonistic interaction between root-knot nematodes *M.*

incognita and the fungus *Pythium graminicola* on sugarcane. Also, *M. incognita* was shown to protect *Phaseolus vulgaris* roots from the fungus *Rhizoctonia solani* (Costa Manso and Huang, 1986).

Papert and Kok (1999) reported that the gelatinous matrix in which eggs of root knot nematodes are deposited provide protection against microbial attack, possibly due to antibiotic compounds from the matrix or from associated bacteria. Orion and Kritzman (1991) studied the antimicrobial activity of gelatinous matrix of *Meloidogyne javanica* and concluded that it has antimicrobial activity probably for the protection of the nematode eggs.

Additivity. It is commonly accepted that *Meloidogyne arenaria* increased the incidence of southern blight of peanut caused by the fungus *Sclerotium rolfsii*. However, Starr et al. (1996) showed in microplots that both pathogens are capable of reducing peanut yield, both reduce population growth of the other, and that no interaction occurs with respect to population growth, yield reduction, or incidence of southern blight. They concluded that the disease complex is due to additive effects of the pathogens on peanut. Whitney (1974) reported that the interaction between *Pythium ultimum* and *Heterodera schachtii* on root-rot of sugar-beet were synergistic but the interaction between *P. aphanidermatum* and *H. schachtii* was only additive. Abawi and Barker, (1984) showed that on tomato roots, necrosis increased with the population density of root-knot nematode *M. incognita*. They suggest that the presence of some soil-borne organisms such as *Fusarium* spp. that are found in association with the roots may increased damage caused by the nematodes, but the interaction was additive. An antagonistic interaction also occurs between the reniform nematode (*Rotylenchulus reniformis*) and the cotton

seedling blight fungus *Rhizoctonia solani* (Sankaralingam and McGawley, 1994). The presence of *R. solani* increased reproduction by *R. reniformis*, but the nematode did not increase cotton seedling blight. The combined effect of the nematode and the fungus inhibited cotton seedling blight compared to plant inoculated with only the fungus.

Migratory Endoparasitic Nematodes: They are the nematodes that enter and migrate within the roots, feeding on various tissues. Feeding and migration of the nematodes damage root tissues resulting in necrotic extensive lesions on the root surface. The best documented example of a synergistic disease complex involving migratory endoparasitic nematodes and fungi is the association between *Pratylenchus penetrans* and *Verticillium dahliae* resulting in the potato early dying (PED) syndrome (MacGuidwin and Rouse, 1990; Powelson and Rowe, 1993). The disease causes premature vine death and declining yields of potato, and is a limiting factor in several potato production regions (Bird, 1981; Wheeler and Riedel, 1994). The effective control of this disease under field conditions with fumigants and nematicides has long suggested the involvement of plant parasitic nematodes. Results of experiments conducted in field microplots clearly showed a synergistic interaction between *P. penetrans* and *V. dahilae* resulting in PED syndrome. No measurable effect on symptom development and potato yield was obtained when *P. penetrans* was present alone, whereas light to moderate effects were observed with *V. dahliae* alone. Also, it was found that other species of *Pratylenchus* interact with *Verticillium* on PED in potato, but at a reduced level. A similar synergistic disease interaction occurs with *F. oxysporium* and *Pratylenchus penetrans* on alfalfa (Mouza and Webster, 1982).

Bowers, et al. (1996) reported that *P. penetrans* increased infection of potato roots by *Verticillum dahliae.* Potato roots were colonized by *V. dahliae* to a significantly greater extent when grown in soil infested with *V. dahliae* and *P. penetrans* than in soil infested with *V. dahliae* alone or with *V. dahliae* and *P. crenatus.* Infection by *V. dahliae* was not observed to be associated with the site of nematode feeding, and it is suggested that the effect of nematodes on initial infection may not be species-specific. It is concluded that the interaction between *V. dahliae* and *P. penetrans* in potato early dying may occur within the root early in the infection process, resulting in an altered or delayed host response to colonization by *V. dahliae.*

Pratylenchus spp. are involved in root-roting complexes. Edmunds and Mai (1966) have shown that the combination between *P. penetrans* and *Trichoderma viride* cause more reduction in root and shoot growth in alfalfa and celery than in either organism alone. Santo and Holtezmann (1970) reported that simultaneous inoculation with *P. zeae* and *P. graminicola* reduced top and root growth more than either organism alone. Although both were present in the same lesion, effects of each appeared independent and additive. The most severe disease developed when plants were inoculated with nematodes seven days prior to inoculation with *P. graminicola.* Hasan, (1988) showed that root rotting symptoms in chrysanthemum roots caused by *Pythium aphanidermatum* and *Rhizocotonia solani* were increased in the presence of *Pratylenchus coffeae* and the interaction among the three organisms was synergistic. Similar results have been shown on maize, where the interactions among *P. brachyurus*, *P. zeae* and the root-rot fungus, *Fusarium moniliforme* were synergistic (Jordaan et al., 1987). LaMondia (1999) showed that *P. penetrans* alone or in combination with the black rot pathogen,

Rhizoctonia fragariae, reduced strawberry yield in microplots over time. The interaction of the two pathogens appeared to be additive rather than synergistic.

Ectoparasitic nematodes: They are nematodes that remain outside the plant and penetrate with only a small portion of their body feeding either on surface tissues or on subsurface tissues. The sting nematodes, *Belonolaimus* spp, cause little damage to the cortex of plants when feeding, but have been associated with increased *Fusarium* wilt incidence in cotton. *Meloidogyne incognita, Hoplolaimus galeatus* and two population of *B. longicaudatus* (NC and GA) were introduced singly and in various combinations with *Fusarium oxysporium*. f. sp. *vasinfectum* on wilt susceptible Rowden cotton. Among the nematodes used, the combination of NC population of *B. longicaudatus* with *Fusarium* promoted the greatest wilt development. The combination of either population of *B. longicaudatus* with *M. incognita* and *Fusarium* induced greater wilt development than comparable inoculum densities of either nematode alone or when *H. galeatus* was substituted for either of these nematodes (Yang et al., 1976). *Belonolaimus gracilis* increased wilt incidence in the wilt susceptible cv. Rowden, and also in cv. Coker 100W, which exhibits a degree of wilt resistance (Holdeman and Graham, 1954).

Mechanisms of Interactions between Nematodes and Fungi

Interactions between nematodes and fungi are often indirect and occur due to induced modifications in the host plant. These interactions commonly result from either wounding or physiological alternations (localized or systemic) in the host plant. Nematode parasitism of plants requires wounding of the hosts, either by simple micropuncture or by rupturing or separating the plant cells (Taylor, 1979). Ectoparasitic nematodes may cause micropunctures on the plant root surface. Migratory endoparasitic

nematodes produce lesions in the root cortex and epidermis. Sedentary endoparasitic nematodes wound the host as second stage juveniles migrate intercellularly through the cortex and establish contact with vascular tissue to induce giant cells or syncytia.

It has been suggested that the ectoparasitic *Belonolaimus longicadatus* has a very long stylet which may provide access to the vascular tissue of the plant to the wilt pathogen, in contrast to *Hoplolaimus galeatus,* which feeds in the cortical tissues. *Fusarium* wilt of cotton was enhanced by the ectoparasitic sting nematode *B. longicadatus,* but not by *H. galeatus* (Prot, 1993). Wounding may function to provide an undefended entry point for the fungus. However, complex anatomical, physiological changes in plant cells are associated with feeding by ectoparasitic nematodes. It is unknown how those changes affect associated parasites. Moreover, wounding likely increases leakage of root contents, which may be an attractants to other pathogens.

Westerlund et al. (1974) observed that *Fusarium oxysporium* f sp. *ciceri* may require wounding for efficient infection of chickpeas. Studies have been done to compare the effects of inoculum levels and wounding on pathogenicity of *F. oxysporium* and *F. solani*. Application of 1×10^6 *F. oxysporium* conidia/ml onto seeds at sowing or onto young seedlings growing in U.C. mix caused disease in only three of 80 plants; these plants wilted and the fungus was recovered from their stems. If seedling roots were trimmed and dipped into conidial suspensions containing from 1×10^4 to 1×10^6 conidia/ml, symptoms developed and the fungus was reisolated. When 1×10^4 to 1×10^6 *F. solani* conidia/ml were applied to intact seeds or nonwounded seedlings, as described above, typical black rot lesions developed. Regardless of the physiological changes that may have been induced by these treatments, the study suggests that *F. oxysporium*

requires a wound for efficient infection whereas *F. solani* does not.

On the other hand, Van Gundy et al. (1977) reported that the development of infection on tomato plants by *R. solani* was delayed by three to four weeks when the fungus was inoculated simultaneously rather than following inoculation with *M. incognita.* The delay in predisposition of plants to fungal diseases by root-knot nematodes suggests that these nematodes are not just wounding agents facilitating the penetration of the fungi within the roots. Rather they showed that increased nutrient mobilization particularly nitrogenous compounds in root leachates three to four weeks after nematode infection, is favourable for maximum virulence of the fungi. When root leachates of plants inoculated simultaneously with the nematode and the fungus were permanently removed, no root-rot occurred. In contrast, when root leachates were not removed a severe root-rot developed. Moreover, when root leachates produced by *M. incognita*-infected plants were applied to roots of plants inoculated with *R. solani* alone, severe root-rot developed, whereas roots inoculated with *R. solani* receiving root leachates from control plants were free of decay.

The mechanism of the interaction between *Pratylenchus* spp. and *Verticillium* spp. is related to the development of necrotic infection courts and biochemical changes in the attacked plants. Necrotic lesions on roots serve as infection courts which facilitate the establishment of the fungus in the court and subsequent invasion (Conroy et al., 1972). *Verticillium dahliae* showed a distinct preference for the lesions on eggplant roots caused by *P. penetrans.* According to Conroy et al., (1972), lesions in tomato roots were more important for fungal invasion than any general physiological changes. The incubation period of *Verticillium* is shortened in *Pratylenchus*-infected plants (Bergeson,

1963; Faulkner et al., 1970). *Pratylenchus minyus* was shown to enhance susceptibility of peppermint to *Verticillium* wilt at the optimum temperature for the nematode (Faulkner and Bolander, 1969). Therefore, physical and physiological changes in the plants infected with *Pratylenchus* are collated together to enhance susceptibility of plants to *Verticillium* wilt.

The mechanism of the interaction between sedentary endoparasitic nematodes and fungi is extensive and complex. For instance, the overall scenario of interactions between root-knot nematodes and *Fusarium* spp. can be visualized in different stages (Walter, 1965; Bergeson, 1972; Cook and Baker, 1983). The initial phase of the interaction occurs in the rhizosphere, where root exudates from root-knot infected plants stimulate the fungal pathogen. The exudates also, suppress the activity of actinomycetes which are antagonists of the wilt fungus. The next phase in these interactions involves the effect of root-knot infection on penetration of wilt fungi. Initially, it was thought that micropunctures caused by nematodes on the plant root facilitated entry of the fungal plant pathogens. Later, it was demonstrated that severity of fungal induced wilt diseases increased when root-knot nematodes were added three to four weeks prior to fungus inoculation of the host in comparison to simultaneous inoculations of both pathogens. This is led to the supposition that the nature of interactions between root-knot nematodes and Fusarium wilt fungi are physiological rather than physical. The ultimate phase of the interactions between the two pathogens occurs during the pathogenesis of the wilt fungus. Modifications of the host plant by root-knot nematodes are the key factor to this phase of interaction leading to increased wilt severity.

Root exudates reflect the biochemical and physiological changes induced by nematode infection, and hence the development of fungi in roots is influenced by the presence of additional metabolic products in the rhizosphere and on the roots. Root exudates, known to attract the motile stage of fungal pathogens (Zentmyer, 1961), represent a source of nutrients for soil microflora, and may be a stimulus for the germination of dormant fungal spores. Thus, change induced by nematodes in the root exudates may be the first stage in the synergistic interaction between nematode and fungi (Taylor, 1990).

Several instances are known in which nematode galled tissue is more susceptible to fungi than non-galled tissue. Innovative work by Golden and Van Gundy, (1975) showed that galled okra and tomato roots infected with *Meloidogyne incognita* in the field and greenhouse were highly susceptible to infection by *Rhizoctonia solani.* Root decay by the fungus occurred 4-5 weeks after nematode infection. The sclerotia of *R. solani* formed only on galled tissue of okra and tomato roots infected with *M. incognita.* A modification of the cellophane-bag technique (Kerr, 1956) was used to study the prepenetration response of *R. solani* to stimuli originating from *M. incognita*-infected and control roots *in situ.* This technique allowed diffusable substances to pass through the membrane, but physically separated the fungus and host. *Rhizoctonia solani* responded to stimuli which originated from *M. incognita*-infected roots and passed through semipermeable cellophane membranes, by forming black sclerotia on the surface of the cellophane membranes directly opposite galls induced by *M. incognita*, while ungalled portions of nematode-infected control roots remained free of sclerotia. The results suggested that the mechanism by which nematode attack predisposed roots to secondary

invasion by *R. solani* was an indirect one. It is hypothesized that the leakage of nutrients from the roots was responsible for attracting the fungus to the galls, and for initiating the sclerotium formation.

This hypothesis supported the work made in 1972 by Golden and Van Gundy which demonstrated that *M. incognita* infected tomato roots start leaking electrolytes 5 days after invasion by the nematode, reaching a maximum level after 3 to 4 weeks. Such exudate-leakage contains more carbohydrates than proteins or amino acids, which increase in concentration only after two weeks or so. These exudates are known not only for enhancing growth of fungi like *R. solani, F. oxysporium* f. sp *lycopersici, Thielaviopsis basicola,* etc, but also have the ability to stimulate germination of the dormant fungal spores present in the soil environment. Also, fungal species like *Trichoderma, Penicillum, Curvularia, Aspergillus*, etc, which are generally considered weak pathogens become significant in the presence of nematode populations.
Khan and Muller, (1982) reported that *Rhizocotonia solani* preferred *M. hapla*-induced galls on radish. The mycelium accumulated over them showed vigorous growth and abundant sclerotial formation. Extensive necrosis of the galls occurred and roots became obliterated. Non-galled regions of roots did not show sclerotial formation.

Wang and Bergeson (1974) suggested that changes in total sugars and amino acids of *M. incognita*-infected root leachates contribute to predisposition of tomato plants to Fusarium wilt. During the first 14 days after nematode infection, when carbohydrates were abundant and the C/N ratio was high in *M. incognita*-infected root leachates, *R. solani* growth was stimulated in the rhizosphere and the fungus was attracted to the roots. Between 14 and 28 days following nematode infection, the C/N ratio decreased and this

low C/N ratio appears to favor parasitic development of *R. solani* (Weinhold et al., 1972).

Although biochemical modifications of root leachates induced by root-knot nematodes appear to enhance the colonization of the rhizosphere by pathogenic fungi, and to attract and favor their growth to gall tissues, they also appear to lower the numbers of actinomycetes, antagonistic to *F. oxysporium* f. sp. *lycopersici*, in the rhizosphere. Bergeson et al. (1970) observed a highly significant reduction of actinomycetes and a significant increase in number of *Fusarium* propagules in the rhizosphere soil surrounding roots inoculated simultaneously with *M. javanica* and *F. oxysporium* f. sp. *lycopersici* compared to those observed when the fungus was inoculated alone. A similar observation was made by Noguera and Smits (1982) who suggested that the reduction in number of actinomycetes antagonistic to *Fusarium* in the rhizosphere of *M. incognita*-infected plants may be partly responsible for enhancement of pathogenic effect of the fungus.

Modifications in hosts that enhance host susceptibly to fungal pathogens may involve systemic physiological changes. These changes render sites removed from the nematode infection more susceptible to the fungus. Adopting split-root techniques, Bowman and Bloom (1966) studied the breaking of *Fusarium* resistance in tomato cultivars Rutgers and Homestead by *M. incognita*. One part of the root system was inoculated with *F. oxysporium* f. sp. *lycopersici* and the other with *M. incognita.* They observed that wilt incidence was increased when the nematodes and the fungus were inoculated on opposite halves of the root system. Similar results were reported for the interaction between *M. incognita* and *F. oxysporium* f. sp. *lycopersici* by El-Sherif and Elwakil (1991) with the tomato cv. Tropic. Carter (1981) reported additive combined

effects of *M. incognita* inoculation on the roots and hypocotyl wounding, facilitating the penetration of *R. solani* on the severity of seedling disease of cotton. Because the two organisms infected spatially separate tissues, this additive effect indicated a localized effect from hypocotyl wounding but a systemic effect from the presence of *M. incognita.*

Sidhu and Webster (1977) studied the translocation of the nematode's predisposing effect by bending over the stems of *Fusarium*-resistant tomato plants (cv. Chico III) four times to produce four adventitious root systems in addition to the primary root system. They inoculated the *M. incognita* on the primary root systems and the fungus *F. oxysporium* f. sp. *lycopersici* on one of the root systems (primary or one of the four adventitious). When the fungus was inoculated on the primary root system, wilt symptoms were observed on the entire plant. When the fungus was inoculated on one of the adventitious root systems, wilt developed at the site of fungal inoculation and on the portion of the plant between the site of fungal inoculation and the apex of stem, whereas, wilt symptoms were minimal between the fungal inoculation site and the base of the plant. These results indicated that a predisposition factor produced or induced by the nematode can be transmitted at considerable distance from the nematode infection site to the upper foliage. Similar results were obtained by Hillocks (1986) in an experiment in which plants growing in *Meloidogyne*-infested soil were stem inoculated with the wilt fungus. The nematode increased wilt severity, despite the physical separation of the two organisms, indicating a systemic effect of the nematodes on the hosts resistance to fungi.

Contradictory results were obtained in split root experiments when the nematode and the fungus were inoculated on opposite parts of the root system (Hillocks, 1986; Moorman et al., 1980). They did not observe any translocatable influence of *M.*

incognita on the development of *F. oxysporium* f. sp. *vasinfectum* and *F. oxysporium* f. sp. *nicotianae* on cotton and tobacco, respectively. However, they observed wilt increase and an enhancement of fungal infection when the nematode and the fungus were together on the same half-parts of the root system. These apparent contradictory results seem to indicate that the nematodes may have two effects favoring fungal infection of their hosts: i) a systemic effect where inhibition of host resistance mechanisms occurs, resulting in a stimulation of the fungal development in tissues not infected by nematodes, and ii) localized effects, where the fungus penetration and initial development in the host is enhanced by the modifications induced by the nematodes at their feeding sites.

Disease Complexes Involving Citrus Nematodes and Fungi

Several studies exist of the associations between nematode parasites of citrus and other microorganisms. Cobb, 1914, was the first to observe that other organisms may be associated with citrus nematode and cause a disease syndrome. The synergistic association between *Tylenchulus semipenetrans* and *Fusarium solani* has been shown by Thomas, 1923. *F. solani* can reduce growth in citrus seedlings under controlled conditions and the growth suppression of citrus seedlings caused by *T. semipenetrans* and *F. solani* together was greater than that caused by either organism alone (Van Gundy and Tsao, 1963). Root decay in lemon by *Fusarium solani* is increased significantly when *T. semipenetrans* is present at 30 °C but not at 20 °C or 25 °C; however, this does not hold true for *F. oxysporium* (O'Bannon et al., 1967). Labuschagne et al. (1989) showed that the combination of *T. semipenetrans* and *F. solani* only affected the growth of citrus trees under field conditions where the average maximum soil temperatures are below 30° C. The effect of both pathogens individually or in combination, would be significantly

greater where trees were subjected to stress caused by conditions such as waterlogging, low quality irrigation water, drought and other adverse conditions.

Effects of Fungus on Nematode Populations

In most synergistic interactions involving nematodes and fungi, usually fungi suppress the development and reduce the population density of the nematode. Interactions between the migratory endoparasitic nematodes such as *Pratylenchus* spp. and pathogenic fungi showed that the population of the nematode usually increases and in some cases decreases. Jordaan et al. (1987) found more root lesion nematodes *Pratylenchus brachyurus* in maize roots infected by *Fusarium moniliforme* which increased attraction and penetration of the nematodes into the roots. Hasan (1988) showed that the reproduction of lesion nematode *Pratylenchus coffeae* on chrysanthemum roots was decreased in the presence of *Pythium aphanidermatum* and increased in the presence of *Rhizoctonia solani*, however when both fungi were present nematode population was unaffected. Santo and Holtzmann (1970) reported that the normal populations of *Pratylenchus zeae* are much lower when *Pythium graminicola* is present than in fungus free roots. In contrast with migratory endoparasities, the sedentary endoparasites such as *Meloidogyne* spp., *Heterodera* spp. and *Globodera* spp. showed reduced population density in the presence of wilt fungi (*Fusarium*, *Verticillum*) and also with root-rot fungi (*Pythium*, *Rhizocotonia* and *Phytophthora*) (Powell, 1971; Hasan, 1984; Gray et al., 1990). Jorgenson (1970) found that the interaction between *Heterodera schachtii* and *F. oxysporium* was antagonistic to the nematode. Damage to sugarbeets was less when the fungus and the nematode were present than when only the nematode was present. The fungus inhibited the nematode invasion and development on

sugarbeet seedlings. Three times more of *H. schachtii* larvae invaded sugarbeet seedlings that were inoculated with nematode only than invaded seedlings inoculated with both organisms. Anwar et al. (1996) showed that greater inhibition of root penetration by *M. incognita,* development of females, galling and nodulation occurred with simultaneous inoculation of *R. solani* and *M. incognita*. Nematode inoculation prior and after fungus treatment resulted in only slight to moderate inhibition of galling, nodulation and female development. Simultaneous inoculation of the fungus with nematodes exhibited a linear decrease in the final population of nematodes in soybean. Sakhuja and Sethi (1986) showed that both *Fusarium solani* and *Rhizoctonia bataticola* have an antagonistic effect on multiplication of *M. javanica*. Both fungi reduced nematode galling. Simultaneous inoculation of one or both fungi in combination with nematode was more inhibitory to galling in comparison to inoculations proceeding or succeeding the nematode. *R. batiticola* inhibited gall formation and nematode multiplication to a greater extent compared to *F. solani.* The effect could be possibly attributed to deleterious effects of metabolites of both fungi on juveniles.

Nematode and Rhizosphere Microbial Community Structure

Many biotic and abiotic factors affect the colonization of roots by microorganisms (Howie, 1985., Howie et al., 1987., Azad et al., 1985., Atkinson et al., 1975). One of the most limiting factors is the indigenous microorganisms, which may enhance or suppress root colonization by another organism (Weller, 1983., Brown, 1981). The presence and the infection by the nematode in the rhizosphere can significantly modify the rhizosphere environment and affect other rhizosphere microorganisms. The nutrient value of the gelatinous egg matrix and leakage from the roots due to the infection by the nematode

may be responsible for increasing or decreasing microbial density around nematode infected roots.

Maloney et al. (1997) reported that successional changes in the composition of rhizosphere microflora occur in response to alteration in root physiology and microbial composition. Bowen and Rovira (1976) showed that nutrients, rather than space, are the limiting factor in competition among rhizosphere microorganisms during colonization, and that bacteria tend to congregate in grooves between cells where nutrients are most abundant. Indigenous microorganisms may also enhance colonization by introduced bacteria (Brown, 1981; Vojinovic, 1973; Weller, 1983). In another case, the population of indigenous, gram-negative bacteria of the *Pseudomonas* spp. were larger on roots infected by *Gaeumannomyces graminis* var *tritici* than on healthy plants (Weller, 1986). Rovira and Wildermuth, (1981) in electron microscopy studies showed that the bacteria proliferate in the lesions, probably owing to the greater availability of nutrients in these micosites. There are many of the rhizosphere bacteria involved in nematode disease complexes (i.e. *Bacillus* spp.; *Burkholderia* spp.; *Arthrobacter* spp.; *Stenotrophomonas* spp.; and others). Some of these bacteria are known as a bicontrol agents against plant pathogenic fungi and also nematodes or function as Plant Growth Promoting Rhizobacteria (PGPR).

Bacillus megaterium forms spores that are resistant to unfavorable conditions, and is a good root colonizer, rhizosphere competitor and remains viable for extended periods, some of the multiple effects of *B. megaterium* on soil microorganisms have been studied (Liu and Sinclair, 1993). *Bacillus megaterium* is considered a potential biocontrol agent for *Rhizoctonia* root rot of soybeans (Zheng and Sinclair, 1996). Al-Rehiayani et al.

(1999) showed that the population densities of *Meloidogyne chitwoodi* and *Pratylenchus neglectus* were reduced up to 50% when potato plants were treated with *B. megeaterium.* Neipp and Becker (1999) showed that two strains of *B. megaterium* reduced nematode infection on sugarbeet when eggs were used as inoculum. Most of the strains they used in the experiment also reduced nematode egg hatch *in vitro*.

Burkholderia cepacia and some other *Bukholderia* strains were given the name "Plant Growth Promoting Rhizobacteria" (PGPR) (Schroth and Hancock, 1981) because of their ability to colonize the roots aggressively and to improve plant growth and preempting the establishment of (or suppressing) (Deleterious Rhizosphere Microorganisms (DRMO) (Suslow, 1982). *Burkholderia cepacia* was used as a biocontrol agent for tomatoes against root diseases caused by *R. solani* and *P. ultimum* (Mao et al., 1998a) reduced *Fusarium* wilt (Larkin-Robert and Fravel-Deborah, 1998). *B. cepacia* reduced corn damping-off caused by species of *Pythium* and *Fusarium* (Mao et al., 1997 and 1998b). *B. cepacia* has been reported to colonize root hairs and enhance their development (De Freitas and Germida, 1990), to produce wide spectrum antifungal metabolites (Lambert et al., 1987), and to protect onion seedlings from damping off disease caused by *F. oxysporium* f. sp. *cepae* (Kawamoto and Lorbeer, 1976). *Burkholderia cepacia* is antagonistic to both *R. solani* and *Pythium* spp. and has been used for biological control of diseases caused by these pathogens (Cartwright and Benson, 1995; King and Parke, 1993; Millus and Rothrock, 1997). Meyer et al. (2001) showed that *B. cepacia* (Bc-2 and Bc-F) and *Trichoderma virens* (Gl-3) significantly suppressed numbers of root-knot nematode eggs and juveniles on roots of pepper plants. *Burkholderia cepacia* (Bc-F) increased shoot dry weights of nematode infected plants,

compared to controls. The combination of the biocontrol agents did not result in a beneficial synergistic interaction, however the agents individually suppressed the nematode populations.

The bacterium *Stenotrophomonas maltophilia* inhibits brown patch disease, caused by *R. solani* (Giesler and Yuen, 1998) and protects sugar beet from *Pythium*-mediated damping-off (Dunne et al., 1998). Single inoculations with either W81 or F113 *S. maltophilia* strains effectively prevented colonization of sugar beet seeds by *Pythium* spp. in soil microcosms. In the field use of both strains as co-inoculated applications proved to be equivalent to the use of chemical fungicides, which improved the protection of sugar beet against *Pythium*-mediated damping-off.

Strains of *Arthrobacter* are capable of causing destruction of *Fusarium* and *Pythium* (Mitchell and Hurwitz, 1964). Lytic *Arthrobacter* isolated from tomato rhizosphere reduced the infection by *Pythium debaryanum* from 88% (when the fungus was alone) to 25% when the fungus and the bacteria were together (Mitchell and Hurwitz, 1964). A single take-all lesion can enhance the population of *P. fluorescens* (Ahmad and Baker, 1987; Howie and Echandi, 1983) 10 fold per centimeter m of root. This is of practical significance, since the infected tissues are where the inhibitory bacteria are needed the most. Colonization of lesion provides considerable protection against secondary spread of take-all fungus on the roots.

Mechanisms by which rhizobacteria exhibit biological control against soil pathogens have been reported (Kloepper, 1993) to include, antibiosis (through bacterial production of antifungal compounds including antibiotics and hydrogen cyanide (Brisbane et al., 1987; Thomashow and Weller, 1988; Howie and Suslow, 1991),

competition for nutrients or for ferric irons (Suslow, 1982; Weller, 1985; Elad and Chet, 1987), competition for infection sites, (Osburn et al., 1989; Baker, 1968) or parasitism and production of lytic enzymes (Mitchell and Alexander, 1961; Mitchell and Hurwitz, 1965; Sneh, 1981).

Plant Growth Promoting Rhizobacteria prevented DRB from colonizing sugar beet because the PGPR occupy and exclude DRB from the cortical cell junctions, at which exudation of nutrients is maximal (Suslow, 1982). Elad and Chet (1987) reported that the suppression of pythial damping-off disease caused by *Pythium aphanidermatum* was correlated significantly to the competition for nutrients between germinating oospores of *P. aphanidermatum* and the biocontrol rhizobacteria. Osburn et al, (1989) showed that *Psudomonas putida* strain R20 that colonized the pericarps, seeds and roots of sugar beet had no effect on germination of *Pythium ultimum* sporangia *in vitro*. However in soil tests the bacteria delayed the fungal colonization of the pericarp 4 to 12 hour after planting, by 24 hour, 90% of untreated seeds were infected with *Pythium*, whereas seeds treated with R20 showed 37% infection. The prevalence of damping off disease was 50% less than control after treatment with R20. They conclude that the disease suppression was due to the protection of the pericarps by occupation of pathogen infection sites by the bacteria. *Arthrobacter* and other rhizosphere bacteria that produce fungal cell wall lytic enzymes have shown a biological control activity against *Pythium* and *Fusarium* spp. However these bacteria did not colonize the roots suggesting that they may not be rhizobacteria but the biological control activity occurred due to parasitism of the fungi (Mitchell and Alexander, 1961; Mitchell and Hurwitz, 1965; Sneh, 1981).

Plant Growth Promoting Rhizobacteria can induce alteration in plant physiology resulting in increased host plant defense to pathogens attack (i.e induced resistance). Wei et al. (1991) showed that PGPR suppressed the anthracnose disease on cucumber caused by *Colletotrichum orbicular* by colonization of roots. The fungus was not detected in petioles or protected leaves suggesting that the antagonism or the competition for disease suppression was due to induced systemic resistance by applied PGPR as a seed treatments. Similar studies by Van Peer et al. (1991) who reported that *Fusarium* wilt of carnation was significantly reduced by root bacterization with *Pseudomonas* strain WC 5417r. The reduction occurs only when plants were bacterized 1 week before stem inoculation with *F. oxysporium* f. sp. *dianthi* and not when they received both treatments simultaneously. The bacterium was not detected in stem suggesting that the disease was suppressed not due to competition but due to induced resistance.

Also, added bacteria may modify the rhizosphere environment and indirectly affect the plant growth. *Bacillus megaterium* and other *Bacillus* spp. known commercially as phosphobacterin (Copper, 1959; Mishustin, 1963) increased the vigor of wheat in the greenhouse but not in the field (Broadbent et al. 1977; Burr et al. 1978). It has been suggested that *Bacillus* spp. enhanced plant growth by different ways (i.e production of biologically active substrates (auxins and gibberellines) or transformation of unavailable minerals and organic compounds and make it available to the plant (Broadbent et al. 1977).

CHAPTER 3

INFECTION OF CITRUS ROOTS BY *TYLENCHULUS SEMIPENETRANS* REDUCES ROOT INFECTION BY *PHYTOPHTHORA NICOTIANAE*

Introduction

Citrus is one of the most economically important crops in many regions with Mediterranean and subtropical climates. The most commonly encountered association between nematodes and fungi that are pathogenic to citrus occurs between *Tylenchulus semipenetrans* Cobb and *Phytophthora* spp. The citrus nematode, *T. semipenetrans,* is distributed throughout all citrus growing regions of the world and causes the disease "slow decline" that results in significant reduction in fruit yield and size (Duncan and Cohn, 1990). The nematode is a semi-endoparasite of the cortical cells of citrus fibrous roots; the female induces several nurse cells surrounding the head in the root cortex, while the posterior part of the nematode, including the egg mass, remains exposed in the soil (Van Gundy, 1958, Cohn 1965). Various species of *Phytophthora* also attack the citrus fibrous root cortex, causing a disease known as fibrous root rot (Graham and Menge, 1999). The most commonly encountered species in the subtropics is *P. nicotianae* Dastur Breda de Haan (synonym = *P. parasitica*) (Graham and Timmer, 1992; Hall, 1993). Fibrous root rot is characterized by soft, water-soaked lesions that expand and quickly result in sloughing of the cortex to leave only the threadlike vascular cylinder. Significant loss of fibrous roots due to infection by *P. nicotianae* can result in less fruit yield and smaller fruit, similar to effects caused by *T. semipenetrans* (Graham and Menge, 1999).

A highly significant inverse relationship between numbers of nematodes following nematicide treatments and numbers of propagules of *P. nicotianae* in the soil was detected in field trials designed to evaluate the effects of nematicides and fungicides on concomitant populations of *P. nicotianae* and *T. semipenetrans* in a citrus grove in Florida (Graham and Duncan, 1997). Because fibrous root density was not increased by nematode management, the relationship between the population densities suggested the possibility that the nematode may inhibit population development of the fungus. We have subsequently investigated the nature of the interaction between *P. nicotianae* and *T. semipenetrans* in a series of field surveys and laboratory studies. In this chapter, we report results of experiments designed to determine whether infection of citrus fibrous roots by *T. semipenetrans* can modulate root infection by *P. nicotianae* and virulence of the fungus to citrus seedlings.

Materials and Methods

***Phytophthora nicotianae* infection of nematode-infected and noninfected root segments in vitro**. Two bioassays were conducted to determine whether infection of roots by *T. semipenetrans* affects subsequent root infection by *P. nicotianae.* Nematode infected citrus roots were collected from naturally infected trees in the field and cut into (2-2.5 mm) segments that were either infected by a single female *T. semipenetrans* or not infected. Segments of each type were surface sterilized for 8 minutes in cupric sulfate (1,000 ppm) and then rinsed five times (500 cm^3 exchange of volume each time) in sterile distilled water. *Phytophthora nicotianae* isolate P-117 (obtained from citrus roots by J. H. Graham at Citrus Research and Education center in Lake Alfred, Florida) was cultured and maintained on *Phytophthora*-selective PARP-H medium, (a cornmeal agar amended

with antibiotics) (Graham, 1990; Mitchell and Kannwischer-Mitchell, 1992). Four agar plugs (5mm diameter) were removed with a cork borer from the margins of actively growing *P. nicotianae* colonies and placed on 2% water agar medium in 100 ×15 mm Petri dishes, equidistance apart. Six nematode-infected root segments were placed 2 mm from each agar plug (24 segments per dish). Each egg mass, exposed on the root surface, was placed in contact with the water agar facing the fungus plug. The same procedure was repeated with root segments not infected by the nematode. Each treatment was replicated six times. Root segments selected were of similar diameter and color. At 4, 7, and 11 days, root segments were removed from two dishes of each treatment (48 segments per treatment) and placed on PARPH media for 72 hour to determine infection by *P. nicotainae.* The experiment was repeated, but segments were evaluated after 12 days exposure to *P. nicotianae*.

Effects of citrus nematode on fungal growth in roots and fungal virulence to citrus seedlings. Two whole-plant experiments were conducted to determine the effect of *T. semipenetrans* on the epidemiology of root infection by *P. nicotianae*. In both experiments, Sour orange (*Citrus aurantium* L.) seeds freshly removed from fruit were air-dried. After seed coats had been removed, seeds were surface sterilized with 10% commercial bleach (0.6% NaOCl) containing 0.01% Tween-20 for 10 minutes and then rinsed five times in sterile distilled water. A single sterilized seed was placed in a 2-cm deep depression made in the center of the surface of autoclaved soil mix (50:50 by volume, Candler fine sand (uncoated, hyperthermic Typic Quartzipsamments and shredded Canadian sphagnum peat moss (Scotts Inc., Sandusky, OH., U.S.A.) in 150 × 25 mm glass test tubes.

In the first experiment, seedlings were inoculated with two levels of the nematode and two levels of the fungus. Eight treatments were established, fungus high, fungus low, nematode high, nematode low, fungus high nematode high, fungus low nematode high, fungus high nematode low, fungus low nematode low.

The second experiment was established in a similar manner, but with different treatments. The pH of the soil mixture for half the plants was adjusted from 4.5 to 7.0 by addition of 3 mL per tube of 10% calcium carbonate, to favor nematode infection. Four factorial treatments were established for each pH level in this experiment: i) nematode infected seedlings, ii) fungus infected seedlings, iii) seedlings infected by both organisms, and iv) seedlings infected by neither organism. Both experiments were run with 10 single plant replicates per treatment, in a completely randomized design arranged in racks in front of a window and maintained at room temperature (25 ± 2 °C), with daily diurnal cycles of light.

Inoculum of *T. semipenetrans* was obtained from naturally-infected roots from the field. Eggs, juveniles, and males were scrubbed from root surfaces and collected on 74/25 μm pore nested sieves. Nematodes were further separated from soil and plant debris by sucrose centrifugation (Jenkins, 1964), then surface sterilized with cupric sulfate (1,000 ppm) for 30 minutes and rinsed five times with sterile distilled water. In the first experiment, a 10 ml mixture of 8,000 or 80,000 eggs and second-stage juveniles of *T. semipenetrans* (low and high inoculum level, respectively) were pipetted into four holes around the stems of each plant in treatments receiving nematodes. In the second experiment, 80,000 nematodes per plant were similarly inoculated. Nematode infection was established for 6 months before *P. nicotianae* treatments were added.

Zoospores of *P. nicotianae* were obtained from colonies of isolate P-117 as described previously. Plugs were placed into sterile 60 ×15 mm Petri plates containing10 ml of sterile half-strength V-8 broth prepared by mixing 110 ml of clarified V-8 juice with 890 ml of water, kept at room temperature in the dark for 4 days for mycelial growth, after which the V-8 broth was decanted and 10 ml of sterile distilled water was added and decanted twice. Plugs were then incubated in 10 ml sterile distilled water 4 days in light at room temperature to produce sporangia. Plates were refrigerated 30 minutes and returned to room temperature to liberate zoospores. Zoospore suspensions were decanted after 45 minutes combined and quantified using a hemacytometer (American Optical Co., New York, NY., U.S.A.). Low and high levels (9,000 and 90,000 zoospores) of fungal inocula in 10 ml water were introduced via canula, 1 to 10 cm deep into the soil in the tubes in the first experiment. Only the high level of zoospores (90,000) was used in the second experiment. Ten milliliters of sterile distilled water was added in the same manner to control tubes.

Six weeks after fungal inoculation, soil was gently rinsed from tubes to remove the plants. Roots were blotted and tap and fibrous roots were separated and weighed. Stem and leaf fresh and dry weights were measured. Root systems from five plants per treatment were blender extracted (Duncan and El-Morshedy, 1996) to estimate the numbers of eggs, second-stage juveniles, and females per gram of root. Roots from the remaining replicates were gently rinsed with tap water and dried for 48 hour in the oven (70 °C), then ground by mortar and pestle. Concentration of *P. nicotianae* protein in 30 mg dried roots was determined using the Agri-screen immunoassay kit (Enyzme Linked

ImmunoSorbent Assay (ELISA) for detection of *Phytophthora* (Neogen Corp., Lansing, MI., U.S.A).

In the first experiment, The data from six balanced factorial treatments (fungus high, fungus low, fungus high nematode high, fungus low nematode high, fungus high nematode low, fungus low nematode low) (all containing the fungus) were analyzed with 2-way ANOVA (Minitab Inc., State College, PA, U.S.A) in which fungus (high or low) and nematode (absent, high or low) were main factors. Data in the second experiment were analyzed by 3-way ANOVA in which pH, fungus and nematode were the main factors.

Results

***Phytophthora nicotianae* infection of nematode infected and non-infected root segments in vitro.** In the first test, approximately twice as many root segments were infected by *P. nicotianae* in the absence of nematodes by 10 days post-exposure (Fig. 3-1) ($P \leq 0.01$). Results of the second test at 12 day post-exposure were consistent with those of the first.

Effects of citrus nematode on fungal growth in roots and virulence to citrus seedlings: In both whole-plant experiments, infection by the citrus nematode reduced the fungal protein (as measured by ELISA) in the seedling roots and increased plant weight compared to plants treated only with the fungus. In the first experiment (Fig. 3-2), levels of fungal protein in the roots infected by both organisms were 53 to 65% lower ($P \leq 0.05$) than in roots infected by only the fungus. Compared to plants infected only by *P. nicotianae*, shoot weights were 33 to 50% greater ($P \leq 0.05$) in plants infected by both parasites. Fibrous and tap root weights were 5 to 23% and 19 to 34% greater ($P \leq 0.05$),

respectively, in nematode fungal combination treatments compared to the fungus alone. There was no significant effect of *P. nicotianae* on *T. semipenetrans* reproduction at any inoculum level. Mean (± standard error) nematode females per gram root was 130 ± 26 in high inoculum treatments and 76 ± 10 in low inoculum treatments. Corresponding means for offspring per gram root were 1,953 ± 158 and 1,178 ± 198 (Table 3-1).

In the second experiment, soil pH affected the growth of seedlings and the population growth of the nematode and the fungus. Root and stem fresh weights of untreated controls were 22% and 40% greater, respectively, when grown at pH 7.0 compared to pH 4.5. The number of nematode females and offspring per gram of roots from plants inoculated only with nematodes were 3.6 and 13.2 times greater, respectively, at high pH compared to low pH (Table 3-1) ($P \leq 0.05$).

Absorbance readings from ELISA plates to detect protein of *Phytophthora* sp. were 72% greater at pH 7.0 than at pH 4.5 for plants inoculated only with *P. nicotianae* (Fig. 3-3) ($P \leq 0.05$). *Phytophthora nicotianae* was the only parasite that reduced seedling mass at either pH. Compared to controls, *P. nicotianae* reduced root fresh weight by 37% at pH 7.0 and 16% at pH 4.5 ($P \leq 0.05$). The fungus reduced stem fresh weight by 27% at pH 7.0 and by 20% at pH 4.5 ($P \leq 0.05$).

Nematodes and fungi each inhibited population growth of the other in some conditions. *Tylenchulus semipentrans* completely suppressed detection of *P. nicotianae* protein in root tissue at both pH levels (Fig. 3-3). At pH 7.0 which favored nematode infection, the fungus had no effect on the final population density of the nematode (Table 3-1). However, in plants grown at pH 4.5, which was less favorable for nematode infection, the fungus reduced the number of nematode females and offspring per gram of

root by 39% and 58%, respectively ($P \leq 0.05$).

With respect to seedling variables, the presence of the nematode completely inhibited disease caused by *P. nicotianae* (Fig. 3-3). Regardless of soil pH, there were no significant differences in stem or root fresh weights in plants infected by both organisms compared to untreated controls (Fig. 3-3). The infection by *T. semipenetrans* increased stem fresh weights (Table 3-2; Fig. 3-3). There was an interaction between the nematode and fungus in terms of root fresh weight ($P \leq 0.005$) and fungus protein in roots ($P \leq 0.001$) (Table 3-2). Further one-way ANOVA revealed that the fungus reduced stem ($P \leq 0.02$) and root ($P \leq 0.002$) weights in the absence of the nematode, but had no effect on stem ($P \leq 0.97$) or root ($P \leq 0.25$) weights in the presence of the nematode. *Phytophthora nicotianae* treatments resulted in increased ($P \leq 0.001$) fungal protein in roots, whereas fungal protein in the *T. semipenetrans* plus *P. nicotianae* treatment did not differ from that in the untreated control (Table 3-2; Fig. 3-3) ($P \leq 0.618$).

Discussion

An antagonistic interaction between *T. semipenetrans* and *P. nicotianae* in these experiments resulted in less infection of roots by the fungus, reduced fungal development in roots, and less growth reduction of citrus seedlings. Although nematode densities among treatments were manipulated by different inoculation rates and different conditions of soil pH, no density dependence in the antagonistic effects was observed for the range of nematode densities achieved in these experiments. For example, manipulation of pH resulted in an approximate 5-fold difference in infection rates by the nematode; however, infection by the fungus and subsequent disease were prevented by

the nematode under both pH conditions. The effect of pH is consistent with results of Van Gundy and Martin (1961) who found higher population densities of *T. semipenetrans* in alkaline than in acid soils. The pH optimum of soil for *T. semipenetrans* development is 6.0-7.5 (Van Gundy et al., 1964).

The consistency of the antagonistic effect in these studies, and observations of significant increases in *P. nicotianae* propagule densities in soil following management of *T. semipenetrans* in a citrus orchard (Graham and Duncan, 1997), suggest that the interaction between this nematode and the fungus is potentially of economic significance.

Our results differ from those of most research involving nematode-fungus interactions. When interactions occur, the nematode frequently plays the primary role as modifier of the host making it more susceptible or more suitable for other pathogens (Pitcher, 1978; Powell, 1979). Interrelationships between plant-parasitic nematodes and soil-inhabiting micoorganisms were first observed by Atkinson (1892) who noted a very close relationship between *Meloidogyne* spp. and *Fusarium* wilt fungi in cotton. The combinations of these pathogens always contribute to more severe losses from wilt than did the fungus alone. Various systems involving sedentary endoparasitic, migratory endoparasitic and ectoparasitic nematode-fungal association are well reviewed (Bergeson, 1972; Holdeman and Graham, 1954; MacGuidwin and Rouse, 1990; Mai and Abawi, 1987; Powell, 1971a, 1971b; Powelson and Rowe, 1993; Prot, 1993). Root-knot and cyst nematodes often predispose plants to more severe infection by other pathogens such as *Fusarium* spp., *Phytophothora* spp., and *Rhizoctonia* spp. (Carter, 1981; McLean and Lawrence, 1993; Powell et al., 1971; Roy et al., 1989; Webster, 1985; Whitney, 1974). Sting nematode, *Belonolaimus* spp., which are ectoparasitic in their feeding habit

cause little damage to the root cortex when feeding, but yet they have been associated with increased *Fusarium* wilt incidence in cotton (Yang et al., 1976). Synergistic disease complexes involving migratory endoparasitic nematodes include the associations between *Pratylenchus penetrans* and *Verticillium dahliae* resulting in the potato early dying syndrome (Bowers, et al., 1996) and *F. oxysporum* and *Pratylenchus penetrans* on alfalfa (Mouza and Webster, 1982).

Antagonism toward plant pathogenic fungi by nematodes is not unknown. Valle-Lamboy and Ayala, (1980) reported an antagonistic interaction between root-knot nematodes *M. incognita* and the fungus *P. graminicola* on sugarcane. The presence of the nematode in combination with the fungus interfered with the fungus development and the plants grew and developed better than when the two microorganisms act separately. Also, *M. incognita* has been shown to protect *Phaseolus vulgaris* roots from the fungus *R. solani* (Costa Manso and Huang, 1986). Gray et al. (1990) found that survival of alfalfa seedlings was reduced following inoculation with *Phytophthora megasperma* f. sp. *medicaginis* compared to inoculation with both *M. hapla* and *P. megasperma.* They suggested that the nematodes feeding on immature roots may have interfered with infection by *P. medicaginis*. An antagonistic interaction also occurs between the reniform nematode (*Rotylenchulus reniformis*) and the cotton seedling blight fungus *Rhizoctonia solani* (Sankaralingam and McGawley, 1994). The presence of *R. solani* increased reproduction by *R. reniformis*, and the combined effect of the nematode and the fungus inhibited cotton seedling blight compared to plant inoculated with only the fungus. The similarity between results of the present study and that of Sankaralingam and McGawley (1994) is noteworthy because both *T. semipenetrans* and *R. reniformis* are

sedentary semi-endoparasites that infect roots by penetrating the mature epidermis and root cortex. This infection process is fundamentally different than that of numerous sedentary endoparasites that penetrate in the rapidly developing zone of elongation and for which antagonistic interactions with fungi are generally unknown. Thus, adaptation of mechanisms to prevent fungal infection of nematode feeding sites may be affected by the mode of root infection by nematodes.

Both *T. semipenetrans* and *P. nicotianae* frequently reside concomitantly in the citrus rhizosphere; therefore, it is not surprising that the nematode may protect its feeding site and may interfere with the fungus either indirectly (through resource competition, alteration of host physiology or alteration of the microbial community in the rhizosphere), or directly (anti-fungal chemicals). *Tylenchulus semipenetrans* was shown to increase the incidence of *Bacillus megaterium* and *Burkholderia cepacia* in the citrus rhizosphere (El-Borai et al., 2000) and both bacteria inhibit a variety of soilborne plant pathogens (Al-Rehiayani et al., 1999; Mao et al., 1997; 1998a, 1998b; Millus and Rothrock, 1997; Zheng and Sinclair, 1996). Eggs of *T. semipenetrans* were recently found to inhibit mycelial growth of *P. nicotianae* and *F. solani* in vitro, in contrast to those of *M. arenaria* (El-Borai Kora et al., 2001). Thus, the nature of the interaction between *T. semipenetrans* and *P. nicotianae* may be complex and additional work on the subject is warranted.

From a practical standpoint, the results of this study suggest that growers need not be concerned that citrus nematode will exacerbate yield or tree losses due to *Phytophthora*-induced root or crown rot. Indeed, infection by the nematode may mitigate damage by the fungus, although field studies are needed to determine whether this

interaction is economically significant and whether this interaction should form a basis for management tactics.

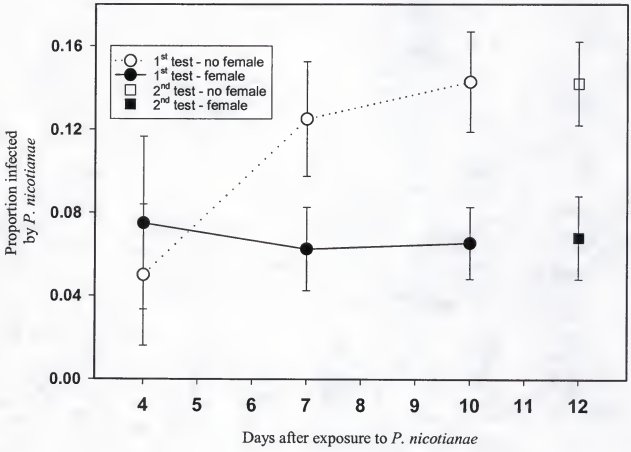

Figure 3-1. *Phytophthora nicotianae* infection of *Tylenchulus semipenetrans* infected and non-infected citrus root segments *in vitro*.

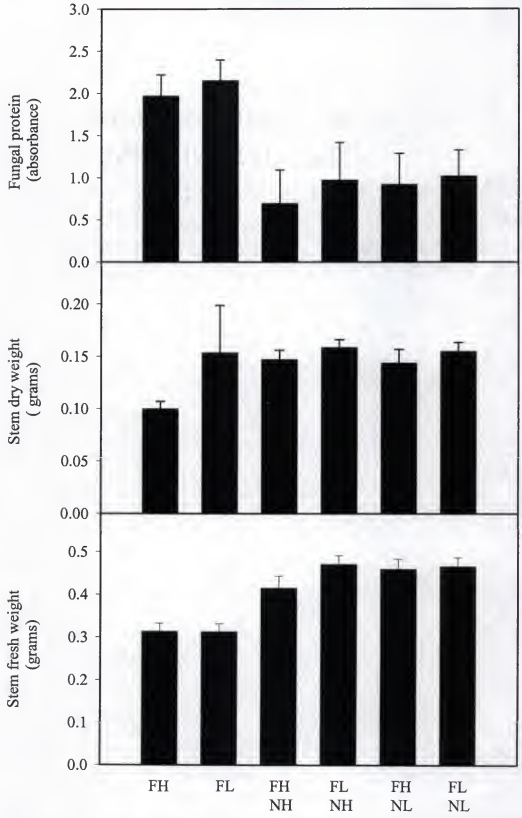

Figure 3-2. Effect of *Tylenchulus semipenetrans* on growth and pathogenicity of *Phytophthora nicotianae* (FH=Fungus High, FL=Fungus Low, NH=Nematode High and NL=Nematode Low). Bars indicate the standard error of the mean for ten seedlings replications per treatment.

Table 3-1. Effect of different soil pH (low = 4.5 and high = 7.0) on rate of reproduction of the citrus nematode *Tylenchulus semipenetrans*.

	Females/g of root		Offspring/g of root	
Treatment	pH 4.5	pH 7.0	pH 4.5	pH 7.0
Untreated control	–	–	–	–
Tylenchulus semipenetrans	121.3 ± 38.2	554.6 ± 110.9	182.5 ± 24	2583.9 ± 562.9
Tylenchulus semipenetrans + *Phytophthora nicotianae*	76.40 ± 2.6*	563.9 ± 152.3	77.7 ± 8.3*	2722.0 ± 688.2

Data values are means of 6 replications ± standard error of the mean.

* Indicates significant difference from control at $P \leq 0.05$.

Table 3-2. Analyses of variance of effects of *Tylenchulus semipenetrans* on stem fresh weight, root fresh weight and *Phytophthora nicotianae* protein in citrus roots in thelaboratory

	Stem weight		Root weight		Fungal protein	
	F-value	P-value	F-value	P-value	F-value	P-value
	Tylenchulus semipenetrans					
PH	37.80	0.000	4.39	0.041	0.64	0.432
Nematode	5.09	0.028	4.93	0.031	22.76	0.001
Fungus	2.55	0.116	1.12	0.295	22.71	0.001
pH × Nematode	0.39	0.534	0.02	0.889	2.05	0.165
pH × Fungus	0.09	0.769	0.25	0.619	2.31	0.141
Nematode × Fungus	2.46	0.122	8.64	0.005	21.75	0.001
pH × Nematode× Fungus	0.64	0.427	1.77	0.190	1.91	0.179

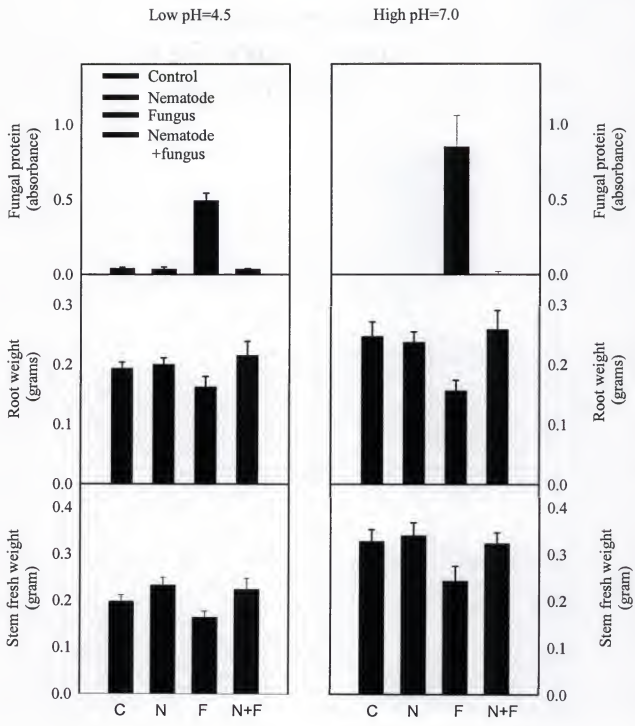

Figure 3-3. Effect of pH and the citrus nematode *Tylenchulus semipenetrans* on growth in roots of *Phytophthora nicotianae* and damage by the fungus to citrus seedlings (C=Control, N=Nematode, F=Fungus, N+F=Nematode plus Fungus). Bars indicate the standard error of the mean for 10 seedlings replications per treatment.

CHAPTER 4

TYLENCHULUS SEMIPENETRANS ALTERS THE MICROBIAL COMMUNITY IN THE CITRUS RHIZOSPHERE

Introduction

The interactions between plant-parasitic nematodes and other plant pathogens are commonly perceived to be indirect, the result of modifications in the host plant such as localized or systemic responses to wounding (Carter, 1981; Hillocks, 1986; Moorman et al., 1980; Westerlund et al., 1974) or other physiological alterations (Bowman and Bloom, 1966; Van Gundy et al., 1977). Root exudates reflecting the biochemical and physiological changes induced by nematode infection alter microbial community dynamics in the rhizosphere and on the roots (Wang and Bergeson, 1974; Weinhold et al., 1972; Van Gundy et al., 1977). Root exudates attract the motile stage of fungal pathogens (Zentmyer, 1961), represent a source of nutrients for soil microflora, stimulate the germination of dormant spores, and may be the first stage in synergistic interactions between nematodes and fungi (Taylor, 1990). For example, root leachates induced by root-knot nematodes (*Meloidogyne* spp.) can enhance the colonization of the rhizosphere by pathogenic fungi (Golden and Van Gundy, 1972, 1975; Kerr, 1956; Khan and Muller, 1982) and reduce the numbers of actinomycetes, antagonistic to other fungi in the rhizosphere (Bergeson et al., 1970). Chemicals that emanate directly from nematodes have been also shown to affect associated microorganisms. The gelatinous matrices surrounding eggs of the root-knot nematodes, *M. javanica* and *M. fallax* provide

protection from microbial attack due either to antibiotic compounds of nematode origin or from associated bacteria (Orion and Kritzman, 1991; Papert and Kok., 1999).

The most common association between pathogens of the citrus fibrous root system is likely that between the citrus nematode *T. semipenetrans* Cobb and the root rotting fungus *P. nicotianae* Breda de Haan (synonym = *P. parasitica* Dastur (Hall, 1993). Both organisms are nearly ubiquitous in citrus growing regions. Each feeds on the cortex of fibrous roots and both have been shown to reduce the density of the fibrous root system (Duncan et al., 1993; Graham and Menge, 1999). Previous work done in the field and greenhouse (Graham and Duncan, 1997) followed by a series of in vitro, laboratory, and greenhouse studies (El-Borai et al., 2000) showed that the citrus nematode *T. semipenetrans* reduced root infection by *P. nicotianae* and increased growth of citrus seedlings compared with seedlings infected by *P. nicotianae* alone. These results imply an antagonistic interaction between *T. semipenetrans* and *P. nicotianae.*

Potential mechanisms for antagonism by nematodes to fungi include direct antibiosis, competition for resources in the roots, or indirect mediation through increased colonization of nematode feeding sites by microorganisms antagonistic to *P. nicotianae.* Following the latter hypothesis, we sought to determine whether infection by *T. semipenetrans* changes the composition of rhizosphere inhabiting microorganisms, and to identify microorganisms that are consistently associated with the nematode. We also conducted in vitro and whole plant experiments with candidate bacteria species to determine their capacity to inhibit root infection by *P. nicotianae* in the presence or absence of citrus nematode.

Materials and Methods

Field survey. A survey was conducted in three citrus orchards to investigate whether root infection by *T. semipenetrans* is associated with changes in communities of bacteria and fungi in the rhizoshere. Roots naturally infected by *T. semipenetrans* were collected from citrus orchards near Ona, Bartow, and Lake Alfred in central Florida. The groves were separated by distances ranging from 18 to 45 miles. Five random trees were sampled in each orchard and five fibrous root samples (0-30 cm-depth) collected from each tree were composited. Six groups of 30 root segments (2.0-2.5 mm length) prepared from each sample were placed in 5-ml distilled water and macerated for approximately 8 seconds in a Tissuemizer (Tekmar Co., Cincinnati, OH., U.S.A). Half of the groups contained root segments infected by female *T. semipenetrans* and the remaining groups contained only uninfected segments as identified at 10× magnification under a stereomicroscope. The resulting suspensions were diluted (10, 10^{-2}, and 10^{-3}) and 0.1-ml of each dilution was streaked on five Petri dishes each of two different media: PARP-H, corn meal agar amended with antibiotics, selective for *Phytophthora* (Graham, 1990; Mitchell and Kannwischer-Mitchell, 1992), and potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, U.S.A). After 72 hours, bacterial and fungal colonies were counted and individual colonies isolated.

Bacteria were cultured on 1.5% nutrient agar (Sigma Chemical Company, St. Louis, MO, U.S.A). The isolates were identified by fatty acid analysis using fatty acid methyl-esters (FAMEs) (Sasser, 1990) and the Aerobic Bacterial Library of MIDI (Microbial Identification, Newark, DE) (Anonymous, 1996). Cultures were maintained at -80° C. Predominant fungal colonies were transferred to new nutrient agar plates

within glass cylinder cells for separation from contaminant organisms by allowing the fungus to grow downward and laterally. The plates were incubated at room temperature (25 °C) for 5 days, after which the procedure was repeated. Fungi were identified to genus or species level by microscopic and sterioscopic analysis, using taxonomic keys.

The survey was conducted twice, first in July 1998 at two sites (Lake Alfred and Ona), when the bacterial and fungal colony forming units (CFUs) were quantified and again in March 1999 at three sites, when CFUs were quantified and identified to species. Data for total bacteria CFUs in both surveys at Lake Alfred and Ona were analyzed by three-way analysis of variance in which site, root type and year were main factors. Data for each microorganism identified in the second survey (3 sites) were analyzed by two-way analysis of variance in which site and root type were main factors.

In vitro inhibition of *P. nicotianae* by isolated bacteria. Bioassays were conducted to determine whether bacteria associated with *T. semipenetrans*-infected roots affect growth of *P. nicotianae*. Bacterial isolates were prepared by streaking them onto nutrient agar and allowing the isolates to grow for 48 hours at room temperature. Using a bacterial loop, a single cell colony from newly grown colonies was transferred to nutrient agar in 100 × 15 mm Petri dishes (Fisher Scientific, Pittsburgh, PA, U.S.A) by streaking the cells in a circle (3-cm wide) circumscribing the center of the dish. A 4-mm mycelial plug taken from an actively growing colony of *P. nicotianae* (isolate P-117 obtained from citrus roots by J. H. Graham at Citrus Research and Education Center, Lake Alfred, Florida) grown in PARP-H media was placed in the center of the dish. Ten replicate-plates for each bacterium were incubated at room temperature for 3 days after which the *P. nicotianae* colony radius was measured from eight different directions and means

determined. Data were subjected to analysis using Dunnett's test (Dunnett, 1955) to compare the control treatment (*P. nicotianae* alone) with the five tested bacteria.

Effects of nematode-associated bacteria on the interaction between *T. semipenetrans* and *P. nicotianae*. Two experiments (greenhouse and laboratory) were conducted to determine whether rhizosphere bacteria in the presence of absence of *T. semipenetrans* affect the virulence of *P. nicotianae* on citrus. Isolates of *Burkholderia cepacia* and *Bacillus megaterium* were used to establish the following incomplete factorial treatments: bacteria alone, bacteria + fungus, bacteria + fungus + nematode, nematode alone, fungus alone, nematode + fungus, and untreated control. All treatments were established under two different conditions of soil pH (low, 4.5 and high, 7.0). Both greenhouse and laboratory experiments were run with ten single plant replicates per treatment, in a completely randomized design.

In the greenhouse experiment, sour orange (*Citrus aurantium* L.*)* seeds freshly removed from fruit were air-dried. Seed coats were removed and seeds were surface-sterilized for 10 minutes with 10% commercial bleach (0.6% NaOCl) containing 0.01% Tween-20, then rinsed five times in sterile distilled water. Sterile seeds were placed individually in 150 × 25 mm autoclaved capped tubes containing MT medium (Murashige and Tucker, 1969) solidified with 0.9% agar (Difco, Detroit, MI, U.S.A) and containing 3% sucrose (pH 6.2) where they germinated aseptically. The culture was maintained at (26 °C ± 2) with 16 hours of cool-white fluorescent light.

Bacterial inocula were prepared (after three weeks storage at –80 °C) by streaking isolated bacteria onto nutrient agar 1.5% in 100 × 15 mm Petri plates and evaluating them for purity after incubation at room temperature for 48 hours. Single cell colonies from

each bacterial culture were transferred to sterile vials with 50 mL Nutrient Broth (Sigma Chemical Co., St. Louis, MO, U.S.A) and incubated with shaking for 36 hours. Cultures were transferred to sterile 50-ml tubes under aseptic conditions and centrifuged at (1000 rpm for 10 minutes) to obtain a bacterial pellet. Pellets were resuspended in 15 mL sterile phosphate buffer (5.8 g Na_2HPO_4 and 3.5 g KH_2PO_4/1000 mL sterile distilled water, pH 7.2). The final suspension absorbance was measured for each bacterium in a spectrophotometer at 620 nm, and adjusted to an average population of 2.4×10^7 per mL. Bacterial suspensions were immediately pipetted into the tubes, 2ml per plant, and 2ml sterile distilled water was added to tubes not receiving bacteria. Three weeks after adding the bacteria, seedlings were transferred to plastic Ray-Leach Containers (1.5-diam. × 8.5 depth) (Stuewe & Sons Inc. Corvallis, OR.,U.S.A) with autoclaved soil mix (50:50 by volume, Candler fine sand (uncoated, hyperthermic Typic Quartzipsammentsand), and shredded Canadian sphagnum peat moss; Scotts Inc., Sandusky, OH, U.S.A). Plants were maintained in the greenhouse (22-24 °C with forteen hours light per day). Ten replications were used for each treatment.

Inoculum of *T. semipenetrans*was obtained from naturally infected field roots. Eggs, juveniles and males were scrubbed from root surfaces by hand rubbing the roots together in water. The nematode life stages were collected on 74/25 µm pore nested sieves. Nematodes were then separated from soil and plant debris by sucrose centrifugation (Jenkins, 1964), surface sterilized with cupric sulfate (1000 ppm) for 30 minutes, and rinsed with five exchanges (500 cm^3 volume each) of sterile distilled water. Two weeks after transplanting the seedlings, a mixture of 90,000 eggs and second-stage juveniles of *T. semipenetrans* were pipetted into four holes around the stems of each plant

in treatments receiving nematodes. Nematodes were permitted to establish on the seedling roots for 6 months before *P. nicotianae* treatments were added.

Zoospores of *P. nicotianae* were obtained by removing nutrient agar plugs from actively growing colonies of *P. nicotianae* (P-117). Plugs were placed into sterile 60 × 15 mm Petri plates containing 10ml sterile half-strength V-8 broth prepared by mixing 110 ml of clarified V-8 juice with 890 ml of water. Plates were incubated in the dark at room temperature 4 days for mycelial growth, after which the V-8 was decanted and 10ml of sterile distilled water was added and decanted twice. Plugs were then incubated in 10-ml sterile distilled water for 4 days in the light at room temperature to produce sporangia. Plates were refrigerated for 30 minutes and returned to room temperature to liberate zoospores. The zoospore suspensions were decanted after 45 minutes, combined and quantified using a hemacytometer (American Optical Co., New York, NY, U.S.A), and 90,000 zoospores in 10mL water were introduced via canula 1 to 10cm deep in soil of appropriate tubes. Ten-milliliter water was added in the same manner to tubes not receiving zoospores.

The laboratory experiment was similarly established with the same treatments as in the greenhouse, except the experiment was conducted in 100-ml glass test tubes into which a single sterile decorticated seed was introduced, as described previously, and placed into the same autoclaved soil mix and allowed to germinate. The soil pH for half the plants was adjusted from 4.5 to 7.0 by addition of 3ml/tube of 10% calcium carbonate to favor nematode infection.

Six weeks after fungal inoculation, soil was gently rinsed from the plastic Ray-Leach Containers or from the glass tubes to remove the plants. Roots were gently blotted

and tap roots and fibrous roots were separated and weighed. Stem fresh and dry weights were measured. Root systems from five plants per treatment were processed (Duncan and El-Morshedy, 1996) to estimate the number of eggs, second-stage juveniles, and females per gram of root. Serial dilutions of macerated root suspensions were made from each treatment. A 300-µl aliquot from each dilution was plated onto nutrient agar to determine numbers of colony forming units (CFUs) of bacteria. Bacteria isolates were identified as described previously. Roots from the remaining replicates were washed free from adhering soil with a minimum of tap water, dried for 48 hours in the oven (70 °C) and ground with a mortar and pestle. Concentration of *P. nicotianae* fungal protein in 30 mg/samples was determined by ELISA test using the Agri-screen *Phytophthora* detection immunoassay kit (Neogen Corp., Lansing, MI, U.S.A). Subsets of the data from both experiments were analyzed by two-way ANOVA (Minitab Inc., State College, PA, U.S.A) of balanced factorial treatments (all those which contained *P. nicotianae*). Main factors were pH and treatments. Population data were transformed ($\log_e n+1$) prior to subjected to ANOVA, but untransformed means are reported.

Results

Field survey. The number of bacteria CFUs from *T. semipenetrans*-infected roots was greater than from uninfected roots in all groves surveyed (Tables 4-1, 4-2). Analysis of variance of repeated surveys for two locations (Lake Alfred and Ona) showed highly significant differences between surveys, groves, and type of root segment (Table 4-1). In the second survey, conducted at three sites, *Bacillus megaterium* and *Burkholderia cepacia* were the dominant bacterial species recovered from nematode-infected and uninfected roots. In each grove *Bacillus megaterium* was recovered from all

sites and *Burkholderia cepacia* was recovered from all but one site. Both species were more numerous on nematode-infected roots than on uninfected roots (Table 4-2). *Arthrobacter ilicis, Stenotrophomonas maltophilia* and *Arcanobacterium haemolyticum* also were recovered from both *Tylenchulus*-infected and uninfected roots in at least one site within each grove. The isolated fungal community was dominated by *Fusarium solani* in each of the three groves (Table 4-2). The 3-way analysis of variance showed no effect of nematode infection in roots on numbers of *F. solani* propagules; however a paired *t*-test of log-transformed data (pairing infected and uninfected root segments from each site within groves) indicated that nematode-infected roots contained more *F. solani* propagules than uninfected roots (df=14; t=3.44; $P \leq 0.004$). Species in the genera *Trichoderma, Verticillum, Phythophthora,* and *Penicillum* also were recovered from at least one sample from each of the groves.

In vitro inhibition of *P. nicotianae* by the isolated bacteria. All isolated bacteria inhibited growth of *P. nicotianae* in vitro compared to the control treatment (Fig. 4-1) ($P \leq 0.05$). There were no significant differences in degree of inhibition among the bacteria.

Effect of nematode associated bacteria on the interaction between *T. semipenetrans* and *P. nicotianae* (laboratory study). Soil pH 4.5 was less favorable for seedling growth in the laboratory experiment compared to pH 7.0. Root and stem fresh weights in the untreated control treatment were 22% and 40% higher, respectively, at pH 7.0 than at pH 4.5 (Figs. 4-2, 4-3). Higher pH also was more favorable for population growth of the bacteria, nematode, and fungus. Total bacteria CFUs were 75% more numerous at pH 7.0 than at pH 4.5 (Fig. 4-4). At pH 7.0 root infection by the nematode

was more than 4-fold ($P \leq 0.05$) that at pH 4.5. Mean ± standard error nematode female per gram root was 554.6 ± 110.9 at pH 7.0 and 121 ± 28.2 at pH 4.5. Corresponding means for offsprings per gram root were 2,583.9 ± 562.9 and 182.5 ± 24.In plants infected only by *P. nicotianae,* fungal protein in roots was 72% greater at pH 7.0 than at pH 4.5 ($P \leq 0.05$).

Phytophthora nicotianae was the only microorganism that reduced seedling root and stem fresh weights at either pH (Table 4-3; Figs 4-2, 4-3) ($P \leq 0.001$). *Burkholderia cepacia* had no significant effect on stem fresh weights but had a highly significant effect ($P \leq 0.01$) on root fresh weights which were 20% higher than those of controls. There were no interactions between this bacterium and *P. nicotianae. Bacillus megaterium* also increased stem (14%) and root (15%) fresh weights, and the bacterium interacted with *P. nicotianae* in this regard ($P \leq 0.001$). Further two-way ANOVA (pH and bacteria) demonstrated that *B. megaterium* had no effect on stem ($P \leq 0.28$) or root ($P \leq 0.10$) weight in the absence of *P. nicotianae*, but that the bacterium increased stem weight by 55% ($P \leq 0.002$) and root weight by 80% ($P \leq 0.001$) in seedlings infected by the fungus compared to fungus only treatment. There were no interactions with pH in either analysis. With regard to *P. nicotianae*, two-way ANOVA (pH and fungus) showed that the fungus reduced the stem weights by 23% ($P \leq 0.001$) and root weight by 28% ($P \leq 0.001$) in the absence of *B. megaterium*, but did not affect stem ($P \leq 0.48$) or root ($P \leq 0.18$) weights in the presence of the bacterium. The fungus effect did not interact with pH for these treatments. Despite the significant effect of *B. megaterium* and *B. cepacia* on the pathogenicity of *P. nicotianae*, neither bacterium affected the amount of fungal protein in the roots (Table 4-3; Fig. 4-5).

As noted previously, (Chapter 3;Table 3-1), the infection by *T. semipenetrans* increased growth of seedlings. *Phytophthora nicotianae* reduced stem and root fresh weight in the absence of *T. semipenetrans* but had no effect on stem and root weight in the presence of *T. semipenetrans.* There were interactions between *T. semipenetrans* and *P. nicotianae* with regard to root weight and amount of fungal protein in roots.

Tylenchulus semipenetrans in combination with *B. cepacia* or *B. megaterium* at pH 7.0, reduced the fungal protein in the roots by 79% and 93% respectively, compared to the fungus only treatment (Fig. 4-5). At pH 4.5 the nematode in combination with either bacteria completely suppressed detection of *P. nicotianae* protein in roots. However, in general the combination of the nematode with either bacterium had few synergistic or additive effects on fungal development in roots or on fungal pathogenicity to seedlings. Three-way ANOVA (pH, nematode, bacterium) of treatments containing *P. nicotianae* showed that the nematode increased shoot and root weight ($P \leq 0.001$) and *B. cepacia* increased root weight ($P \leq 0.007$), but not shoot weight ($P \leq 0.19$). There were no interactions between the nematode and bacterium ($P \leq 0.87$, and $P \leq 0.97$, respectively), suggesting an additive effect on root weight. The same analysis for *B. megaterium* showed that the bacterium increased shoot ($P \leq 0.004$) and root weights ($P \leq 0.001$) of fungus-infected plants and demonstrated interactions between the bacterium and the nematode for both shoot ($P \leq 0.006$) and root weight ($P \leq 0.002$). Further two-way ANOVA (pH, nematode) revealed that the nematode increased shoot ($P \leq 0.001$) and root weights ($P \leq 0.001$) in the absence of the bacterium, but not in its presence ($P \leq 0.29$, and $P \leq 0.18$, respectively).

In plants infected by *P. nicotianae*, the addition of *T. semipenetrans* increased the number of bacteria CFUs ($P \leq 0.003$) at both pH levels, regardless of inoculation with *B. cepacia* or *B. megaterium* (Fig. 4-4). An interaction occurred with pH for effect of the bacterium on the nematode population density. *Burkholderia cepacia* doubled numbers of nematode females and offspring ($P \leq 0.001$ and 0.004, respectively) in plants treated with *T. semipenetrans* and *P. nicotianae* at pH 4.5; however, the bacterium reduced ($P \leq 0.04$) nematode offspring by 73% and did not affect females ($P \leq 0.41$) at pH 7.0. In plants infected by *P. nicotianae* and *T. semipenetrans*, *B. megaterium* increased numbers of female (320 vs 532; $P \leq 0.01$) and offspring (1,399 vs 1,690; $P \leq 0.02$) nematodes at both pH levels.

Effect of nematode associated bacteria on the interaction between *T. semipenetrans* and *P. nicotianae* (greenhouse study). *Burkholderia cepacia* interacted with *P. nicotianae* in terms of stem and root weights (Table 4-4). Further one-way ANOVA showed that the fungus reduced the stem (36%; $P \leq 0.003$) and root weights (39%; $P \leq 0.001$) in the absence of *B. cepacia*, but did not affect stem ($P \leq 0.75$) or root ($P \leq 0.11$) weights in the presence of the bacterium. Similarly, *B. cepacia* increased stem (36%; $P \leq 0.02$) and root weights (39%; $P \leq 0.03$) in the presence of the fungus, but not in its absence ($P \leq 0.95$ and 0.31, respectively). In contrast to the laboratory experiment, *B. megaterium* had no effect on stem ($P \leq 0.603$) weight. However, results were consistent with the laboratory experiment for root weights, where *B. megaterium* interacted with *P. nicotianae* ($P \leq 0.013$). Further one-way ANOVA showed that the fungus reduced the root weight (38%; $P \leq 0.001$) in the absence of *B. megaterium* but did not affect the root weight ($P \leq 0.23$) in the presence of the bacterium. Similarly, *B.*

megaterium increased root weight by 39% ($P \leq 0.06$) in the presence of the fungus, but not in its absence ($P \leq 0.11$). As in the laboratory, neither *B. cepacia* nor *B. megaterium* affected the amount of *P. nicotianae* protein in roots (Table 4-4).

The average (± standard error) nematode female per gram of root was 14.6 ± 3.6, and offspring per gram of root was 5.8 ± 3.01. Although the nematode density was very low, the *P. nicotianae* protein in roots was 60% less in plants infected by both nematode and fungus than in plants infected by only the fungus (Fig. 4-5). Root and stem fresh weights were 39% and 25% greater ($P \leq 0.05$), respectively, in plants infected by both nematode and the fungus compared to plants infected by *P. nicotianae* alone (Figs. 4-2, 4-3). There was interaction ($P \leq 0.068$) between *T. semipenetrans* and *P. nicotianae* with respect to root ($P \leq 0.048$) and stem ($P \leq 0.06$) fresh weights (Table 4-4). One-way ANOVA showed that stem and root fresh weights in fungal infected plants were increased by 23% ($P \leq 0.05$) and 28% ($P \leq 0.0001$), respectively, by the presence of *T. semipenetrans*. *Phytophthora nicotianae* protein in roots infected by both the fungus and nematode was less than half that in roots infected by only the fungus (Fig. 4-5), but in contrast to the laboratory experiment the effect was not significant (Table 4-4).

In plants infected by *P. nicotianae* and *B. cepacia*, *T. semipenetrans* increased bacterial propagule numbers (Fig. 4-4) ($P \leq 0.005$). There was no effect of *B. cepacia* treatment on numbers of *T. semipenetrans*. Treatment with nematodes reduced the number of bacteria CFUs in seedlings treated with *B. megaterium* and *P. nicotianae* ($P \leq 0.06$). In contrast to the laboratory experiment, *B. megaterium* reduced ($P \leq 0.046$) nematode offspring in plants treated with *P. nicotianae* and *T. semipenetrans* but did not affect the number of nematode females.

Discussion

Tylenchulus semipenetrans altered the microbial community in the citrus rhizosphere by increasing propagule densities of bacteria and fungi in each of the three groves studied. All of the isolated bacteria suppressed growth of *P. nicotianae* in vitro; however, in contrast to *T. semipenetrans*, no bacteria inhibited growth of the fungus when inoculated in whole-plant experiments. Nevertheless, the nematode and both of the selected bacteria increased the growth of citrus seedlings infected by *P. nicotianae.* These results suggest that multiple mechanisms may attenuate of virulence of *P. nicotianae* in roots infected by *T. semipentrans.*

The positive effect of *T. semipentrans* and *P. nicotianae* on population increase of rhizosphere microorganisms likely results from leakage of nutrients from fungus-induced root lesions, nematode infection sites, or gelatinous egg masses. Our results agree with Weller, (1986) who reported that densites of indigenous, gram-negative bacteria of *Pseudomonas* spp. were greater on roots infected by *Gaeumannomyces graminis* var *tritici* than on healthy plants. Rovira and Wildermuth (1981) used electron microscopy to show that bacteria proliferate in fungus-induced lesions. Bergeson et al., (1972) showed a significant increase of *Fusarium* propagules in the rhizosphere of roots inoculated simultaneously with *M. javanica* and *F. oxysporium* f. sp. *lycopersici* compared to roots inoculated with only the fungus.

The dominant bacterial species isolated in these surveys, *B. megaterium* and *B. cepacia,* are well documented biological control agents (Liu and Sinclair, 1993; Schroth and Hancock, 1981; Mao et al., 1998; Zheng and Sinclair, 1996). Both species have been described as " plant growth promoting rhizobacteria" (PGPR; Schroth and Hancock, 1981) because of their ability to improve plant growth by aggressively colonizing roots

and preempting the establishment of deleterious rhizosphere microorganisms (Suslow et al., 1982). *Bacillus megaterium* forms endospores that are resistant to unfavorable conditions. The bacterium is a good root colonizer, rhizosphere competitor, and remains viable for extended periods (Liu and Sinclair, 1993). Multiple effects of *B. megaterium* on soil microorganisms have been documented (Liu and Sinclair, 1993) and the bacterium has been shown to be a potential biocontrol agent for *Rhizoctonia* root rot of soybeans (Zheng and Sinclair, 1996). *B. cepacia* has been reported to colonize and enhance root hair development (De Freitas and Germida, 1990) and to produce wide spectrum antifungal metabolites (Lambert et al., 1987). The bacterium has been studied for biological control of diseases caused by many plant pathogenic fungi on different crops such as *R. solani*, *Pythium ultimum* (Mao et al., 1998) and *F. oxysporum* (Larkin-Robert and Fravel-Deborah, 1998) on tomato, and damping-off diseases caused by species of *Pythium* and *Fusarium* (Mao, et al., 1997, 1998) on corn seedlings and *F. oxysporium* f. sp. *cepae* on onion seedlings (Kawamoto and Lorbeer, 1976). PGPR also can alter plant physiology and increase the host plant defenses to pathogen attack (induced resistance). Colonization of roots by PGPR suppressed anthracnose of cucumber leaves caused by *Colletotrichum orbicular* (Wei et al, 1991). Root bacterization with *Pseudomonas* strain WC 5417r reduced the incidence of fusarium wilt in carnation and the amount of *F. oxysporium* f. sp. *dianthi* in stems of the plants (Van Peerr et al., 1991).

The suppressive effect of *B. cepacia* and *B. megaterium* on growth of *P. nicotianae* in vitro is in agreement with Turney et al. (1992) who noted that all bacterial isolates collected from citrus rhizosphere soil inhibited growth of *P. nicotianae* on agar

plates. However, no general relationship exists between the ability of a bacterium to inhibit a pathogen in vitro and suppress disease caused by the pathogen *in vivo* (Baker, 1987; Schroth and Hancock, 1981). Despite their potential as biological control agents, neither bacteria in this study showed evidence of suppressing the rate of infection or growth of *P. nicotianae* in citrus seedlings. Therefore, the results of the whole plant experiments suggest that these bacteria may increase tolerance of citrus seedlings to infection by *P. nicotianae.* Both bacteria increased weights of citrus seedlings infected by *P. nicotianae*, and in general the fungus did not significantly affect weights of the roots or stems of seedlings that were treated with either bacteria. We did not investigate causes of bacteria-induced seedling tolerance to *P. nicotianae.* Possible mechanisms by which PGPR can enhance plant tolerance are poorly understood, but may involve favorable modifications to the rhizosphere chemistry. *Bacillus megaterium* and other *Bacillus* spp. formulated commercially as Phosphobacterin (Copper, 1959; Mishustin, 1963) increased the vigor of wheat in the greenhouse but not in the field, possibly by transformation of unavailable minerals and organic compounds or by production of biologically active substrates such as auxins or gibberellins (Broadbent et al., 1977; Burr et al., 1978).

The effect of *T. semipenetrans* on growth of *P. nicotianae*-infected seedlings in the greenhouse was consistent with those in the laboratory experiment which were reported previously (El-Borai et al., 2000). The general lack of additive or synergistic effects by combinations of bacteria and nematodes is not surprising since treatment with either bacteria or nematodes tended to result in normal seedling growth similar to that of untreated controls. Moreover, these experiments were not controlled to the extent that

involvement by these two bacteria in the effects demonstrated by the nematode treatment can be discounted. All treatments with *T. semipenetrans* increased microbial populations and both bacteria were encountered in all treatments by the end of the experiment. However, unlike either bacterium, all treatments with nematodes reduced the amount of *P. nicotianae* protein detected in roots. This is the only direct evidence from these experiments for a possible mechanism by which pathogenicity of the fungus is attenuated by the presence of another organism. Eggs of *T. semipenetrans* were shown recently to be inhibitory to the growth of *P. nicotianae* (El-Borai Kora et al., 2001). Therefore, there is a good likelihood that infection of citrus roots by *T. semipenetrans* reduces population growth of *P. nicotianae* by direct antibiosis, which mitigates virulence of the fungus. All *T. semipenetrans*-induced changes in the microbial community revealed in this study appear to be favorable to the citrus root system; however, whether augmentation of natural levels of bacteria by nematodes has a significant effect on the plant is unknown.

The effects of *B. cepacia* on *T. semipenetrans* offspring at higher pH were consistent with Meyer et al. (2001) who showed that *B. cepacia* (Bc-2 and Bc-F) and *Trichoderma virens* (Gl-3) significantly suppressed numbers of root-knot nematode eggs and juveniles on roots of pepper plants. *Psudomonas aureofaens* inhibited *Criconemella xenoplax* egg hatch in vitro and reduced nematode population densities in the greenhouse (Westcott and Kluepfel, 1993). The effect of *B. megaterium* on *T. semipenetrans* in the greenhouse experiment was consistent with Neipp and Becker (1999) who showed that two strains of *B. megaterium* inhibited hatching *Heterodera schachtii* from cysts in vitro and reduced nematode numbers and infection of sugarbeet seedlings when eggs were used as inoculum. However the bacterium did not affect *H. schachtii* root infection in

growth pouches. Treating potato plants with *B. megeaterium* reduced population densities of *Meloidogyne chitwoodi* and *Pratylenchus neglectus* by up to 50% (Al-Rehiayani et al., 1999). Nevertheless, both bacteria increased numbers of *T. semipenetrans* under some of our experimental conditions. Differences in nematode species, or concomitant infection of the nematode with *P. nicotianae*, may account for variable effects of the bacteria on *T. semipenetrans* and other nematode species.

In conclusion, these studies indicate that infection of citrus roots *by T. semipenetrans* increases population densities of rhizosphere microorganisms, some of which may increase the tolerance of citrus seedlings to infection by *P. nicotianae.* However, we found no evidence that reduced infection of roots by the fungus in the presence of *T. semipenetrans* is mediated by changes in the rhizosphere microbial community. Additional studies that better control the combinations of nematodes, fungi, and bacteria, by excluding background contamination, are needed to demonstrate a direct effect of the nematode on suppression of root infection by the fungus. Field studies are also warranted to determine if disease caused by *P. nicotianae* is reduced by the nematode and whether the effect is economically important. Nevertheless, this study provides further evidence that *T. semipenetrans* is unlikely to exacerbate fibrous root rot of citrus caused by *P. nicotianae* (El-Borai Kora et al., 2001).

Table 4-1. Total of bacteria colony forming units isolated from root segments infected and uninfected by *Tylenchulus semipenetrans* collected from two different locations.

	1998 Survey		1999 Survey	
	Infected	Uninfected	Infected	Uninfected
Grove Location				
Lake Alfred	21615 ± 1922	8597 ± 611	21500 ± 2182	9955 ± 600
ONA	2390 ± 349	855 ± 104	8405 ± 564	4875 ± 902
	F-value	P-value		
ANOVA				
Year	28.09	0.001		
Grove	115.02	0.001		
Root type[a]	24.03	0.001		
Year × grove	23.00	0.001		
Year × root type	00.01	0.911		
Grove × root type	00.05	0.830		
Year × grove × root type	00.16	0.690		

Numbers are the transformed mean ± standard error of five replications from each grove in each survey. Data were log transformed prior to ANOVA.

[a] Roots were infected or uninfected by *Tylenchulus semipenetrans*.

Table 4-2. Numbers of colony forming units of *Bacillus megaterium*, *Burkholderia cepacia*, and *Fusarium solani* isolated from root segments infected and uninfected by *Tylenchulus semipenetrans* collected from three different locations.

	Bacillus megaterium		*Burkholderia cepacia*		*Fusarium solani*	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
Grove Location						
Lake Alfred	13279.6 ± 1630.2	5964.7 ± 797.5	4744.4 ± 867.5	1911.9 ± 358.8	316 ± 60.2	36 ± 12.7
ONA	5901.5 ± 481.5	4081.5 ± 840.3	1275.0± 100.2	633.5 ± 106.5	964 ± 300.3	364 ± 128.3
Bartow	5808.0 ± 651.5	4140.0 ± 550.4	1440.6 ± 196	544.0 ± 93.20	19.2 ± 5.1	4.8 ± 1.5

	F-value	P-Value	F-value	P-value	F-value	P-value
ANOVA						
Grove	2.73	0.089	1.39	0.271	5.06	0.015
Root type[a]	3.41	0.079	4.44	0.047	1.82	0.190
Grove × root type	0.99	0.389	0.44	0.650	0.32	0.727

Numbers are the untransformed mean ± standard error of five replications from each grove. Data were log transformed prior to ANOVA.

[a] Roots were infected or uninfected by *Tylenchulus semipenetrans*

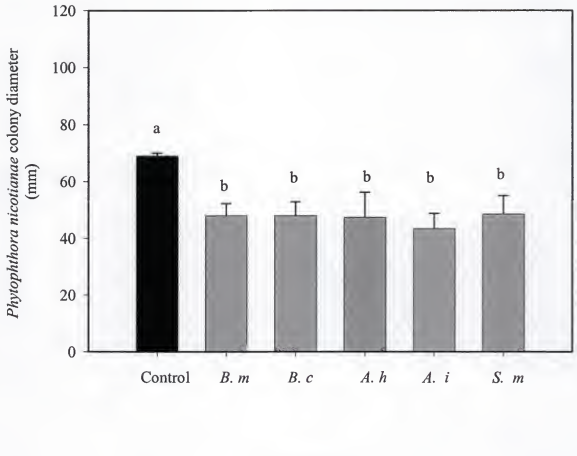

Figure 4-1. Effect of nematode-associated bacteria on *Phytophthora nicotianae* mycelial growth in vitro. Bars followed by a common letter are not significantly different according to Dunnett test ($P \leq 0.05$). *B. m= Bacillus megaterium, B. c=Burkholderia cepacia, A. h= Arcanobacterium haemolyticum, A. i=Arthrobacter ilicis, S. m=Stenotrophomonas maltophilia.*

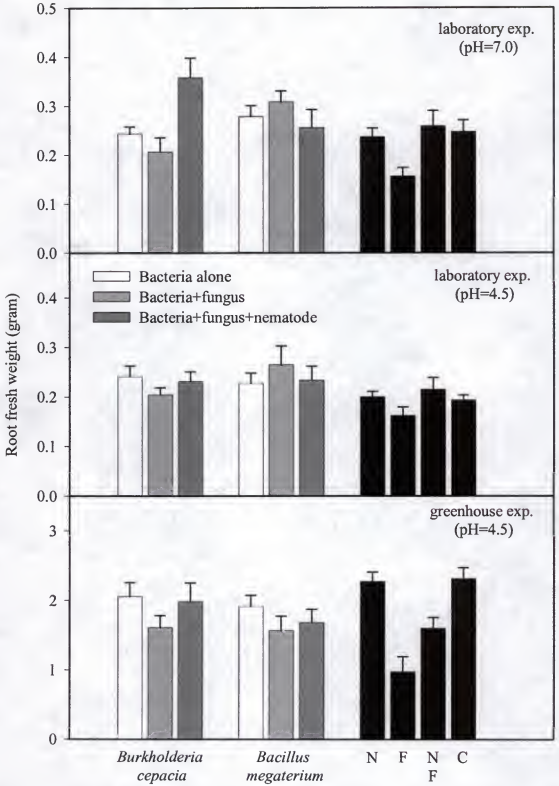

Figure 4-2. Effect of *Burkholderia cepacia, Bacillus megaterium* and *Tylenchulus semipenetrans* on citrus seedlings growth and virulence of *Phytophthora nicotianae* to seedlings root fresh weight. Bars indicate the standard error of the mean for eight seedlings replications per treatment. N=Nematode, F=Fungus, NF=Nematode+Fungus, and C=Untreated control.

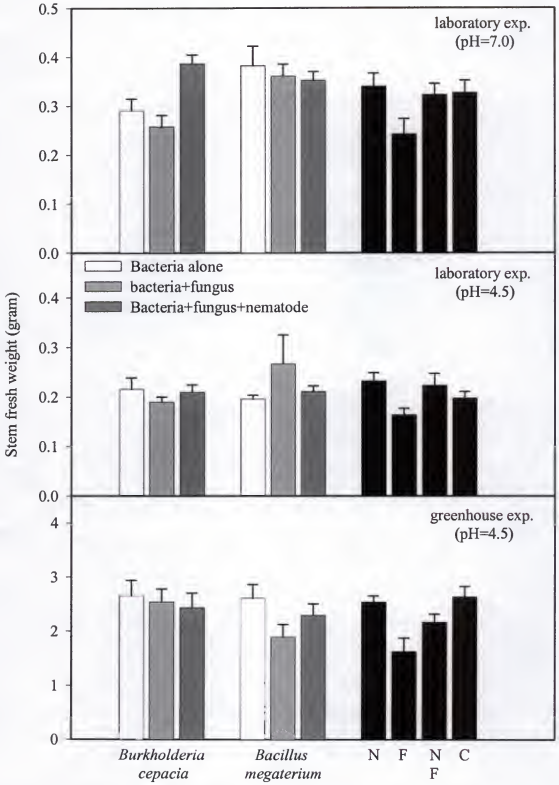

Figure 4-3. Effect of *Burkholderia cepacia, Bacillus megaterium,* and *Tylenchulus semipenetrans* on citrus seedlings growth and virulence of *Phytophthora nicotianae* to seedlings stem fresh weight. Bars indicate the standard error of the mean for eight seedlings replications per treatment. N=Nematode, F=Fungus, NF=Nematode+Fungus, and C=Untreated control.

Table 4-3. Analyses of variance of effects of *Burkholderia cepacia,* and *Bacillus megaterium,*on stem fresh weight, root fresh weight, and *Phytophthora nicotianae* protein in citrus roots in the laboratory.

	Stem weight		Root weight		Fungal protein	
	F-value	P-value	F-value	P-value	F-value	P-value
			Burkholderia cepacia			
pH	33.20	0.000	0.90	0.347	4.27	0.049
Fungus	8.60	0.005	12.60	0.001	20.80	0.000
Bacteria	0.16	0.693	6.12	0.016	1.50	0.230
pH × Fungus	0.90	0.346	1.19	0.281	2.36	0.136
pH × Bacteria	1.15	0.287	0.60	0.443	1.17	0.290
Fungus × Bacteria	0.94	0.337	0.74	0.392	0.16	0.697
pH × Fungus × Bacteria	0.51	0.478	1.18	0.283	0.06	0.802
			Bacillus megaterium			
pH	37.50	0.000	5.72	0.020	2.40	0.134
Fungus	0.76	0.388	0.83	0.367	25.80	0.000
Bacteria	11.90	0.001	28.90	0.000	0.35	0.557
pH × Fungus	3.18	0.080	1.28	0.262	2.75	0.110
pH × Bacteria	0.83	0.366	0.63	0.430	0.23	0.639
Fungus × Bacteria	4.42	0.040	10.10	0.002	0.30	0.589
pH × Fungus × Bacteria	0.26	0.609	0.78	0.382	0.07	0.796

Table 4-4. Analyses of variance of effects of *Burkholderia cepacia, Bacillus megaterium*, and *Tylenchulus semipenetrans* on stem fresh weight, root fresh weight and *Phytophthora nicotianae* protein in citrus roots in the greenhouse.

	Stem weight		Root weight		Fungal protein	
	F-value	P-value	F-value	P-value	F-value	P-value
			Burkholderia cepacia			
Fungus	5.51	0.230	22.90	0.001	22.25	0.001
Bacteria	3.76	0.059	1.04	0.314	0.46	0.504
Fungus × Bacteria	3.44	0.070	5.76	0.021	2.09	0.159
			Bacillus megaterium			
Fungus	14.06	0.001	19.50	0.001	9.09	0.005
Bacteria	0.27	0.603	0.26	0.614	2.66	0.114
Fungus × Bacteria	0.39	0.536	6.81	0.013	0.03	0.860
			Tylenchulus semipenetrans			
Nematode	1.70	0.199	3.15	0.082	2.16	0.155
Fungus	16.38	0.002	36.90	0.001	11.27	0.003
Nematode × Fungus	3.48	0.068	4.10	0.048	2.25	0.146

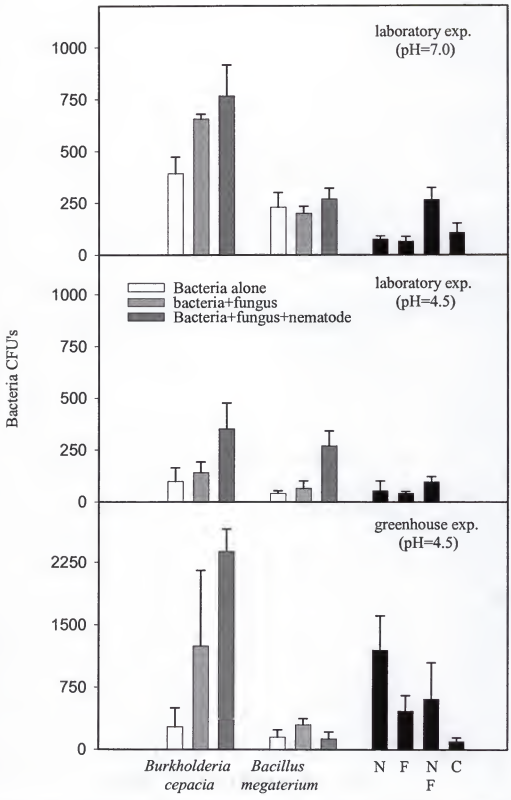

Figure 4-4. Numbers of bacteria colony forming units recovered from citrus seedlings. Bars indicate the standard error of the mean for five seedlings replications per treatment. N=Nematode, F=Fungus, NF=Nematode+Fungus and C=Untreated control.

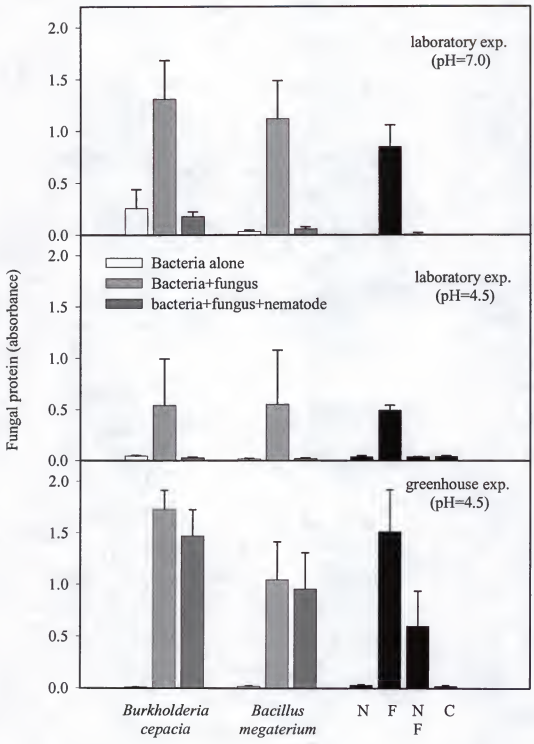

Figure 4-5. Effect of *Burkholderia cepacia, Bacillus megaterium,* and *Tylenchulus semipenetrans* on absorbance of *Phytophthora nicotianae* protein in citrus roots (measured by ELISA test). N=Nematode, F=Fungus, NF=Nematode+Fungus and C=Untreated control.

CHAPTER 5

EGGS OF *TYLENCHULUS SEMIPENETRANS* INHIBIT GROWTH OF *PHYTOPHTHORA NICOTIANAE* AND *FUSARIUM SOLANI* IN VITRO

Introduction

Citrus nematode, *Tylenchulus semipenetrans* Cobb, is a semi-endoparasite of the cortical cells of citrus fibrous roots. The anterior portion of the female extends several cell layers deep in the cortical parenchyma, while the posterior portion, outside of the root, secretes a gelatinous matrix into which eggs are deposited (Van Gundy, 1958). The eggs with this protective gelatinous matrix is known as an egg-mass (Maggenti, 1962). Egg masses contain up to 75-100 eggs (Baines, 1950). The female of *T. semipenetrans* is sessile, obtaining its nutrients from specialized transfer cells (6 to 10) called "nurse" cells around the nematode head (Van Gundy, 1958). These "nurse" cells are required for successful reproduction and die upon the female's death.

Interactions between the citrus nematode *T. semipenetrans* and fibrous root rot fungus *Phytophthora nicotianae* Dastur Breda de Haan (synonym = *parasitica*) (Hall, 1993) in citrus have been shown to be antagonistic to the fungus (El-Borai et al., 2000). Citrus seedlings infected by the nematode and later inoculated with the fungus, grew larger and contained less fungal protein in the root tissues than plants not infected by the nematode. Both organisms feed in the cortex and have been shown to reduce the mass of the fibrous root system (Duncan et al., 1993) and impact citrus yield (Duncan et al., 1993; Graham and Menge, 1999).

Interactions involving nematodes and fungi have been studied extensively and are often synergistic (Atkinson, 1892; Bergeson, 1972; Carter, 1981; MacGuidwin and Rouse, 1990; McLean and Lawrence, 1993a, 1993b & 1995; Mai and Abawi, 1987; Powell, 1971a, 1971b; Powell et al., 1971; Powelson and Rowe, 1993; Prot, 1993; Roy et al., 1989; Whitney, 1974; Webster, 1985). Only occasionally are the interactions antagonistic (Valle-Lamboy and Ayala, 1980., Costa Manso and Huang, 1986., Gray et al., 1990., Sankaralingam and McGawely, 1994). Orion and Kritzman (1991) and Papert and Kok (1999) reported that the gelatinous matrix of the root-knot nematode, *Meloidogyne javanica* and *Meloidogyne fallax* provide protection against microbial attack either due to antibiotic compounds from the matrix or due to associated bacteria.

Possible mechanisms by which the citrus nematode suppresses fungal development include direct chemical antagonism by the nematode, nutrient competition, or alteration of the microbial community in the rhizosphere to favor microorganisms antagonistic to *P. nicotianae.* The objective of this study was to determine the effect of *T. semipenetrans* eggs on *P. nicotianae* and *Fusarium solani* in vitro compared to eggs of the root-knot nematode *M. arenaria.*

Materials and Methods

Bioassays were conducted to determine the effect of eggs of two nematodes, *T. semipenetrans* and *M. arenaria* on *P. nicotianae* and *F. solani* mycelial growth in vitro in three different experiments. In the first experiment, *Tylenchulus semipenetrans* inoculum was obtained from naturally-infected roots from the field. Eggs, juveniles and males were scrubbed from root surfaces and collected on 74/ 25 μm pore nested sieves. Nematodes were further separated from soil and plant debris by sucrose centrifugal-

flotation method (Jenkins, 1964) followed by magnesium sulfate fractionation. The magnesium sulfate (225.9g/liter water) solution was underlayed beneath nematode suspensions, then centrifuged for three minutes at 1,500 rpm (Hendrickx et al., 1976). The interface containing the nematodes was drawn off using a 5-mL pipet. Nematodes were rinsed repeatedly with tap water over a 635 μm pore sieve to remove residual magnesium sulfate. To separate eggs from vermiform stages, a 325 μm pore sieve was used to retain the vermiform stages (as well as free living nematodes). Approximately 15 to 20 passes of the nematode suspension in greater than 1,000 volumes of water was sufficient to purify eggs. The egg suspension was then concentrated on a 20-μm-pore sieve. The few remaining free-living nematodes in the suspension were hand picked.

In the second experiment, eggs of *T. semipenetrans* were scrubbed from root surfaces using 5% commercial bleach (0.03% NaOCl) for 30 seconds. Bleach was used to ensure that no residue of the matrix remained on eggs.

In the Third experiment, an isolate of *M. arenaria* (Neal) Chitwood race 1 from Levy County, Florida was used. This isolate was cultured on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in steam-pasteurized potting soil. The eggs were extracted either by scrubbing the galled tomato roots gently in 5% commercial bleach (0.03% NaOCl) for 30 seconds (Hussey and Barker, 1973; McClure et al., 1973) and caught on a 25 μm pore sieve) or with the same procedure but without using bleach. The same magnesium sulfate and sieving procedures described for *T. semipenetrans* were used to remove debris and separate the *M. arenaria* eggs from juveniles and other nematodes.

Eggs in all experiments were surface-sterilized in a laminar flow hood. Nematode eggs were back-washed into 12-ml sterile disposable plastic tubes. The egg suspension

was allowed to settle for 1 hour and volume was reduced to 0.5 ml of egg suspension per tube using a sterilized 5-ml pipet. Three treatments were used in each experiment conducted: live-surface sterilized eggs, heat-killed surface-sterilized eggs (60 °C for 10 minutes), and a water control. Both live and heat-killed surface sterilized eggs were treated with cupric sulfate (0.1%) for 30 minutes; mercuric chloride (0.025%) for 10 minutes; and then streptomycin sulfate (0.2%) for 24 hours. The eggs were rinsed seven times on an autoclaved 25-μm-pore sieve with 1-liter exchanges of sterile distilled water between each sterilant.

Approximately, 35,000 eggs of each nematode in a 5-μL water droplet were deposited in the center of nutrient agar in 100 × 15 mm Petri plates. Nutrient agar plugs (5-mm diam.) cut with a cork borer from margins of actively growing colonies of either *P. nicotianae* or *F. solani* fungal isolates were placed on the agar surface over the eggs and incubated at room temperature. Control plates received 5-μL sterile distilled water in place of nematode eggs. After 48 hours, fungal colony growth diameter was determined by means of linear measurements made in eight directions starting from the center of the fungal plug using a template made from an inverted Petri dish placed under the dish and measurements averaged. Each treatment was replicated 15 times (one plate per replicate).

To independently test the effects of the sterilization chemicals on fungal growth, a series of dilutions (0, 10^{0}, 10^{-2}, 10^{-3}, and 10^{-4}) were made of the original concentrations of each compound used to surface-sterilize the eggs. Five μL from each dilution were pipetted onto the center of nutrient agar, and a plug of *P. nicotianae* was then placed on top. After 48 hours, *P. nicotianae* colony growth diameter was measured as described

previously. Eight plates were used with each dilution, for each chemical sterilant. Both live and heat-killed eggs of both nematode species were observed for hatching in a 60 × 15-mm Petri plate for a 1-month period. Approximately 1,000 eggs of each nematode species from each treatment in 3-ml sterile distilled water were counted weekly.

All experiments were conducted twice. Data were analyzed by one-way Analysis of Variance (ANOVA), and mean separation was determined with Duncan's multiple range test at ($P \leq 0.05$).

Results

Surface-sterilized live *T. semipenetrans* eggs inhibited *P. nicotianae* mycelial growth by 74% compared to water controls and 72% compared to heat-killed eggs (Figs. 5-1A; 5-2A) ($P \leq 0.05$). In a repeated experiment (Fig. 5-1B) with the same treatments, live as well as heat-killed surface-sterilized eggs were inhibitory and both reduced *P. nicotianae* colony growth by 94% in both treatments compared to controls. *Tylenchulus semipenetrans* eggs extracted with bleach did not differ in their activity against *P. nicotianae* mycelial growth compared to eggs extracted without bleach (Fig. 5-1C). Live surface-sterilized *T. semipenetrans* eggs extracted without bleach inhibited *F. solani* mycelial growth by 92% compared to controls and heat-killed eggs (Figs. 5-3A; 5-2B). In the repeated experiment, both the live and heat-killed surface-sterilized eggs of *T. semipenetrans* inhibited *F. solani* mycelial growth and reduced colony diameter by 95% compared to water controls and 55% compared to heat-killed eggs (Fig. 5-3B; $P \leq 0.05$). With bleach extraction, both live and heat-killed eggs inhibited *F. solani* colony diameter by 94% ($P \leq 0.05$) compared to water controls after 36 hours (Fig. 5-3C). However, heat-killed eggs lost some of their inhibitory activity compared to live eggs after 72

hours. Live *T. semipenetrans* eggs inhibited *F. solani* colony growth by 79% compared to 55% inhibition from the heat-killed eggs (Fig. 5-3C).Live *M. arenaria* eggs had no comparable effect on *P. nicotianae* mycelial growth (Fig. 5-5A) the effect of *M. arenaria* eggs on *P. nicotianae* was the same with or without bleach extraction (Fig. 5-4A). The effect of *M. arenaria* on *F. solani* mycelial growth contrasted with the effect on *P. nicotianae*. Live *M. arenaria* surface-sterilized eggs extracted with bleach did not inhibit *F. solani* mycelial growth (Figs. 5-4B; 5-5B) compared to controls; but without bleach, the mycelial growth of *F. solani* was inhibited by 64% compared to water controls (Fig. 5-4B).

The original concentrations of 0.025% mercuric chloride and 0.2% streptomycin sulfate, each individually inhibited *P. nicotianae* mycelial growth by 100% and 66%, respectively, compared to water controls. However, there were no significant differences in mycelial growth with any other dilutions of either sterilant compared to water controls (Table 5-1). Cupric sulfate had no significant effect on the mycelial growth of *P. nicotianae*. When all three surface sterilants were mixed together, the original concentration was the only treatment that had a significant inhibitory effect on *P. nicotianae* colony growth (100%) compared to all other dilutions and water controls (Table 5-1) ($P \leq 0.05$).

Sixty-five percent of surface-sterilized *T. semipenetrans* eggs hatched compared to 75% of eggs rinsed with water only. Seventy-five percent of surface-sterilized *M. arenaria* eggs hatched compared to 90% of eggs rinsed with water only. No juveniles hatched from heat-killed eggs in any experiment.

Discussion

The results of these experiments demonstrated a direct, species-specific effect of citrus nematode eggs on *P. nicotianae* and *F. solani* mycelial growth. Though the heat-killed eggs of *T. semipenetrans* in the repeated experiment showed an inhibitory effect on *P. nicotianae* and *F. solani*, the more consistent inhibition of live eggs suggests that eggs may actively secrete compounds that inhibit fungal growth. It is unlikely that the disinfectants used to surface-sterilize the eggs caused these effects since a one hundred-fold dilution of the original concentration of these compounds was sufficient to alleviate any fungal inhibition. Eggs were rinsed seven times with 1-L exchanges of sterile distilled water between each sterilant, to demonstrate that the inhibition effect on *P. nicotianae* was due to a direct effect of the eggs and not the disinfectants.

We showed previously that the interaction between *T. semipenetrans* and *P. nicotianae* is antagonistic to the fungus (El-Borai et al., 2000). *Tylenchulus semipenetrans* interfered with *P. nicotianae,* reducing levels of infection in roots and producing increased growth of citrus seedlings compared with seedlings infected by *P. nicotianae* alone. This study suggests that *T. semipenetrans* eggs secrete antifungal compounds that inhibit the development of *P. nicotianae* in roots. Similarly, the gelatinous matrix of root-knot nematode has been shown to provide protection against microbial attack (Orion and Kritzman, 1991; Papert and Kok, 1999). However, this is the first study to demonstrate inhibition of fungal mycelial growth by nematode eggs. Inhibition by *T. semipenetrans* eggs of *P. nicotianae* and *F. solani* with or without bleach extraction suggests that the effect is likely due to chemicals secreted by eggs and is not contained in the gelatinous matrix which is dissolved and removed by the bleach. Conversely, *M. arenaria* eggs extracted without bleach did inhibit *F. solani* mycelial

growth whereas those extracted with bleach did not, suggesting that the residual gelatinous matrix may contain constitutive compounds that inhibit the growth of certain fungi. Differences in the effects of eggs of root-knot and citrus nematodes on plant pathogenic fungi may result from adaptation to different parasitic behaviors. Because the female root knot nematode remains sessile inside the developing root tissue for most of its life (Bird, 1962), this species may experience less selection pressure to protect its feeding site compared to the semi-endoparasitic *T. semipenetrans* that infects and remains exposed on portions of the roots that already fully developed (Cohn, 1965).

Nematode fungal interactions are often synergistic. There are few examples of antagonistic interactions between nematodes and fungi. Gray et al. (1990) found that survival of alfalfa seedlings was lower following a single inoculation with only *Phytophthora megasperma* f. sp *medicaginis* than following inoculation with both *Meloidogyne hapla* and *P. megasperma* f. sp *medicaginis*. The root-knot nematodes *Meloidogyne incognita* have been shown to interfere with the development of the fungus *Pythium graminicola* on roots of sugarcane (Valle-Lamboy and Ayala, 1980). The presence of the nematode in combination with the fungus interfered with the fungus development on roots, and the fungus partially reduced the detrimental effect of the nematode. The antagonistic interaction between both organisms was beneficial to the plants which grew and developed better when both were together than when the two microorganisms act separately. Also, *M. incognita* has shown to protect *Phaseolus vulgaris* roots from the fungus *Rhizoctonia solani* (Costa Manso and Huang, 1986). Sankaralingam and McGawely (1994) also reported an antagonistic interaction between the reniform nematode *Rotylenchulus reniformis* and the cotton seedling blight fungus

Rhizoctonia solani. The combined effect of the nematode and the fungus was antagonistic, with respect to cotton seedling blight. Because *T. semipenetrans* and *R. reniformis* are both sedentary semi-endoparasites with similar life histories, it would be interesting to test activity of eggs of *R. reniformis* for activity against other plant pathogenic fungi.

Tylenchulus semipenetrans eggs had an antagonistic effect on *P. nicotianae* mycelial growth in vitro. These results may explain *T. semipenetrans*-mediated suppression of root infection by *P. nicotianae* that resulted in increased citrus seedling growth relative to seedlings infected with the fungus alone (El-Borai et al., 2000). Further work is warranted to identify and characterize the compounds secreted by eggs of *T. semipenetrans* that inhibit the growth of *P. nicotianae*.

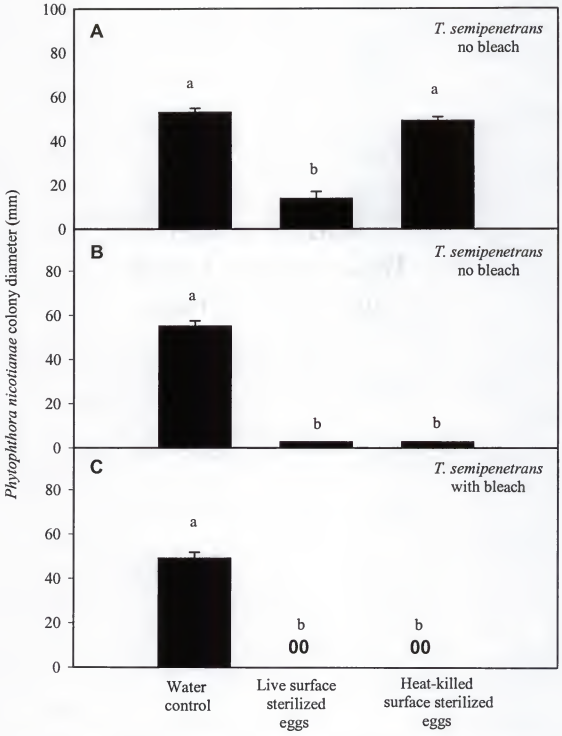

Figure 5-1. Effect of *Tylenchulus semipenetrans* eggs on *Phytophthora nicotianae* mycelial growth after 48 hour *in vitro*. A, B and C are the results of the three experiments. Bars indicate the standard error of the mean for 15 replications per treatment. Bars followed by a common letter are not different according to Duncan's multiple range test ($P \geq 0.05$).

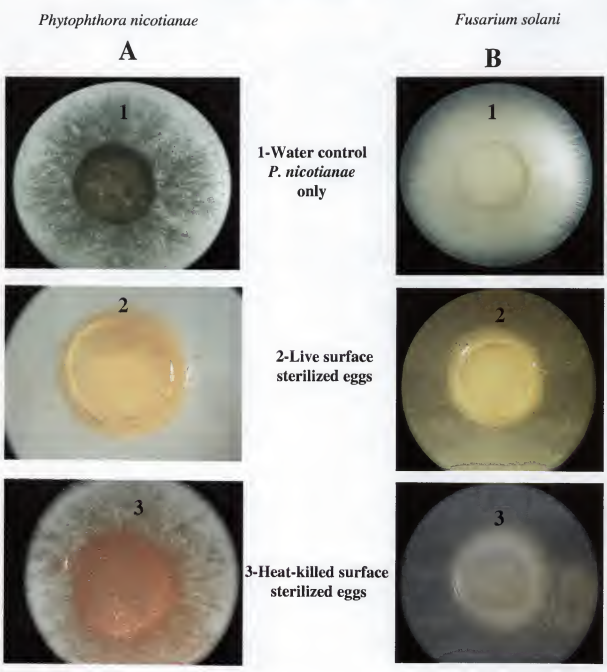

Figure 5-2. Effect of *Tylenchulus semipenetrans* eggs on *Phytophthora nicotiane and Fusarium solani* mycelial growth after 48 hr *in vitro*. Eggs were extracted without bleach. A1 B1= Water control (*Phytophthora nicotianae* only), A2B2 =*Tylenchulus semipenetrans* live surface sterilized eggs, A3B3= *Tylenchulus semipenetrans* heat-killed surface sterilized eggs.

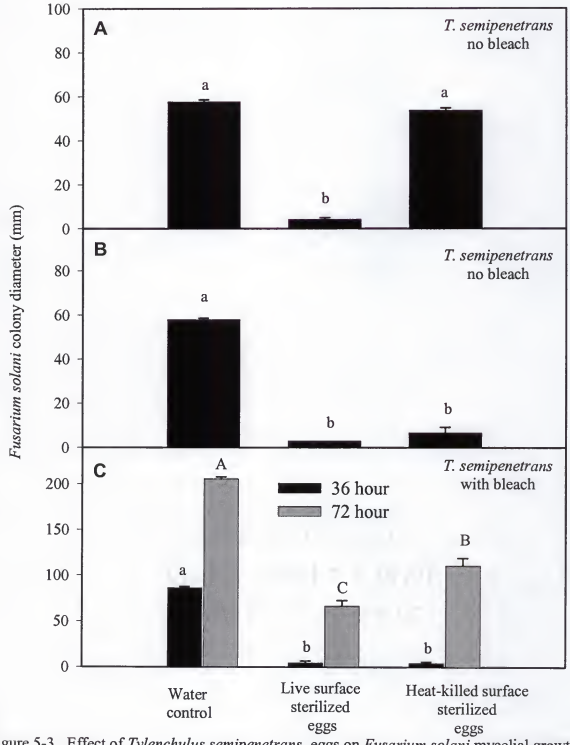

Figure 5-3. Effect of *Tylenchulus semipenetrans* eggs on *Fusarium solani* mycelial growth after 48 hr in A+B and 36 +72 hour in C *in vitro* . A and B are the results of two replicate experiment. Bars indicate the standard error of the mean for 15 replications per treatment. Bars followed by a common letter are not different according to Duncan's multiple-range test ($P \geq 0.05$).

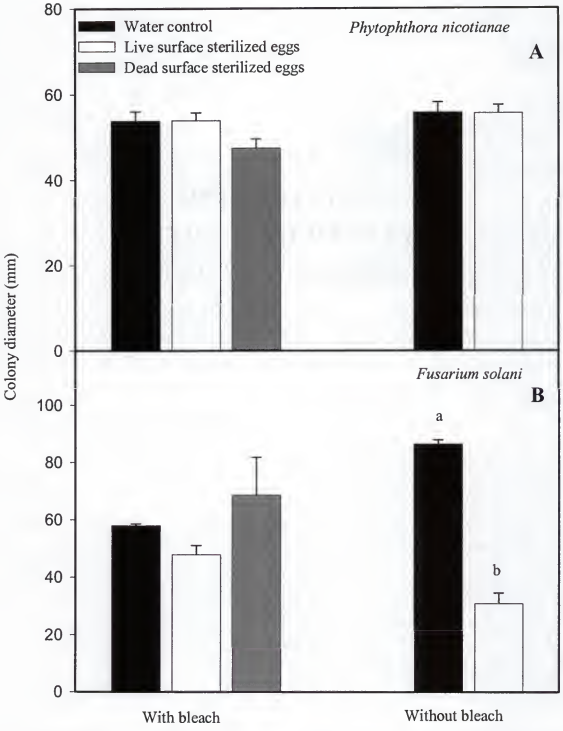

Figure 5-4. Effect of *Meloidogyne arenaria* eggs (extracted with and without bleach) on *Phytophthora nicotianae* (A) and *Fusarium solani* (B) mycelial growth after 48 hour *in vitro*. Bars indicate the standard error of the mean for 15 replications per treatment. Bars followed by a common letter within each experiment are not different according to Duncan's multiple range test at ($P \leq 0.05$).

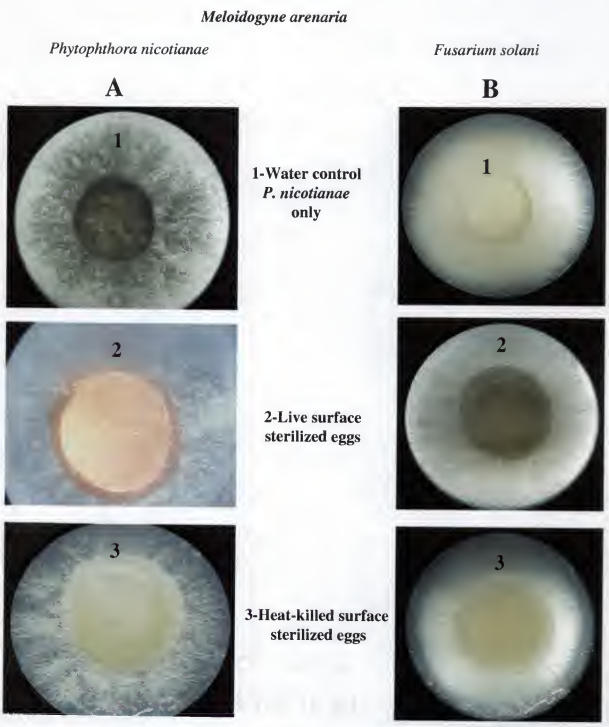

Figure 5-5. Effect of *Meloidogyne arenaria* eggs on *Phytophthora nicotianae* and *Fusarium solani* mycelial growth after 48 hr *in vitro*. Eggs were extracted with bleach. A1B1=Water control treatment (*Phytophthora nicotianae* only, A2B2=*Meloidogyne arenaria* live surface sterilized eggs, A3B3= *Meloidogyne arenaria* heat-killed surface sterilized eggs.

Table 5-1. Effect of cupric sulfate, mercuric chloride and streptomycin sulfate on *Phytophthora nicotianae* mycelial growth after 48 hours in vitro.

	Phytophthora nicotianae colony diameter (μm)			
Dilutions	Cupric sulfate	Mercuric chloride	Streptomycin sulfate	Cupric sulfate Mercuric chloride Streptomycin sulfate
00	57.79 a	57.79 a	57.79 a	22.36 a
10^{0}	59.04 a	29.00 b	39.40 b	0.00 b
10^{-2}	59.79 a	55.92 a	58.02 a	23.11 a
10^{-3}	60.77 a	57.77 a	56.17 a	23.13 a
10^{-4}	63.75 a	59.02 a	58.71 a	23.38 a

Numbers are the means of eight replications for each dilution. Numbers in a column followed by a common letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

CHAPTER 6
RESEARCH SUMMARY AND CONCLUSIONS

Plant parasitic nematodes can play a major role in disease interactions involving a variety of other organisms. Interactions involving nematodes often have important economic effects on the survival and growth of plants and they contribute substantially to variability in crop growth. The most common association between plant parasitic nematodes and fungi in citrus is that between citrus nematode *Tylenchulus semipenetrans* and fibrous root rot fungus *Phytophthora nicotianae.* Both parasites occupy the fibrous root cortex and both are economically important pathogens. There are few data reporting the influence of these two pathogens on one another; however, a recent study found that chemical suppression of citrus nematode was related to increased density of *P. nicotianae* propagules in citrus orchards. The present research was initiated to test whether the nematode does in fact suppress *P. nicotianae* and to investigate the mechanism(s) by which such an interaction might occur.

A series of *in vitro* bioassays and whole plant experiments were conducted to investigate the nature of the interaction between both organisms. A bioassay revealed that fibrous root segments containing *T. semipenetrans* females and egg masses were subsequently infected by *P. nicotianae* only half as often as were uninfected root segments. In whole plant experiments conducted in the laboratory and greenhouse, citrus seedlings infected by the nematode and later inoculated with the fungus grew larger and contained less fungal protein in the root tissues than did plants infected by only the

fungus. Results of this series of experiments demonstrated a significant interaction between *T. semipenetrans* and *P. nicotianae* in which the nematode is antagonistic to the fungus.

Possible mechanisms by which the nematode might inhibit root infection by the fungus include direct antibiosis, resource competition, and indirect mediation by microorganisms associated with nematode feeding sites. Following the later hypothesis, a field survey was initiated to investigate whether *T. semipenetrans* alters the composition of rhizosphere inhabiting microorganisms, and to determine whether microorganisms associated with the nematode affect the behavior of *P. nicotianae*. In each of three citrus orchards the numbers of bacteria colony forming units from *T. semipenetrans*-infected roots were greater than those from uninfected roots. *Bacillus megaterium* and *Burkholderia cepacia* were the dominant bacterial species recovered and both were more numerous on nematode-infected roots than on uninfected roots. *Arthrobacter ilicis, Stenotrophomonas maltophilia and Arcanobacterium haemolyticum* were commonly encountered at lower propagule density. The fungal community was dominated by *Fusarium solani,* but *Trichoderma, Verticillum, Phythophthora*, and *Penicillum* were also recovered. All isolated bacteria inhibited growth of *P. nicotianae in vitro*. Experiments were then conducted using combinations of selected bacteria (*Bacillus megaterium* and *Burkholderia cepacia*), citrus nematode, and *P. nicotianae*, under different conditions of soil pH designed to result in different infection levels by the nematode. Soil pH of 4.5 reduced the infection rate of the nematode and also the fungus and reduced growth of seedlings, and amount of bacterial colonization. Bacteria CFU's (not identified to species) were recovered in significantly greater numbers in plants

nicotianae and *T. semipenetrans* at both pH levels. Root and stem fresh weights of fungus-infected plants treated with *T. semipenetrans*, alone or in combination with *B. cepacia* or *B. megaterium* were greater than for the plants treated with *P. nicotianae* only. *Phytophthora nicotianae* did not negatively affect seedling growth in the presence of either bacterium, whether alone or in combination with *T. semipenetrans*. However, neither bacterium suppressed the amount of *P. nicotianae* protein in citrus roots when used alone in a treatment. All treatments with *Tylenchulus semipenetrans* reduced the fungus protein in roots. These studies showed that nematode-induced changes in the rhizosphere community of bacteria may mitigate the virulence of *P. nicotianae*, perhaps by increasing plant tolerance, but not by inhibiting infection by the fungus. The results suggests that the "biological control" attributed to plant growth promoting rhizobacteria PGPR (Schroth and Han cock, 1981; Wei et al., 1991; Van Peer et al., 1991) may be less important than the effects these organisms have on the ability of plants to grow in the presence of pathogens.

I then studied the possibility of direct inhibition of the fungus by the nematode *in vitro* bioassays were developed to determine the effects of eggs of two nematodes, *T. semipenetrans* and *Meloidogyne arenaria*, on *P. nicotianae* and *Fusarium solani* mycelial growth. Three treatments were used; live-surface sterilized eggs; heat-killed surface-sterilized eggs; and a water control. Live citrus nematode eggs suppressed mycelial growth of *P. nicotianae* and *F. solani* consistently, compared to heat-killed eggs and water controls. Root-knot nematode eggs had no comparable effect on mycelial growth of either fungus. The experiment demonstrated a species-specific, direct effect by the citrus nematode on the behavior of *P. nicotianae* and *F. solani.*

It is not surprising that *T. semipenetrans* may have adapted to protect its feeding site from a ubiquitous and sympatric pathogen of the citrus fibrous root cortex. In contrast to other nematode parasites of citrus, the citrus nematode has a narrow host range that includes citrus and only a few other woody perennials. It has the most refined and complex host-parasite relationship of any nematode parasite of citrus. The nematode is a good parasite, being relatively less virulent to citrus than other nematode parasites of the genus. These characteristics are consistent with the hypothesis that *T. semipenetrans* and its host (citrus) have co-evolved to a significant degree (Duncan, 1995). Indeed, selection pressure may favor a relatively benign response of the plant to infection by the nematode, because mechanisms that prevent infection of citrus roots by fungal pathogens would confer an adaptive advantage not only to the nematode, but to its host.

This appears to be the first report of growth inhibition of a plant parasitic fungus by a plant parasitic nematode. These results suggest that, unlike many nematode fungus disease complexes, *T. semipenetrans* is unlikely to exacerbate plant damage caused by *P. nicotianae* in citrus. Indeed, in orchards infested with *P. nicotianae*, concomitant infection of citrus roots by *T. semipenetrans* may change the rhizoshpere chemistry and microbial community in ways that favor development of citrus roots. *T. semipenetrans* eggs inhibited two different classes of fungi, Oomycetes (*P. nicotianae)* and Hyphomycetes (*F. solani),* but had no negative effect on bacterial colonization of roots, thus exhibiting some selectivity toward fungi. Identification and characterization of the agent(s) in *T. semipenetrans* eggs that is responsible for *P. nicotianae* inhibition may provide a new class of fungicide for management of *P. nicotianae.* Identification of the chemical(s) may also facilitate identification of the nematode genes responsible for the

effect and may lead to a new means of introducing resistance against *P. nicotianae* into susceptible rootstocks.

LITERATURE CITED

Abawi, G. S., and K. R. Barker. 1984. Effects of cultivar, soil temperature, and population levels of *Meloidogyne incognita* on root necrosis and Fusarium wilt of tomatoes. Phytopathology 74:433-438.

Ahmad, J. S., and R. Baker. 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77:182-189.

Al-Rehiayani, S., S. L. Hafez, M. Thornton, and P. Sandararaj. 1999. Effects of *Pratylenchus neglectus*, *Bacillus megaterium*, and oil radish or rapeseed green manure on reproductive potential of *Meloidogyne chitwoodi* on potato. Nematropica 29:37-49.

Anonymous, 1996. Sherlock microbial identification system operating manual, version 6. Newark, DE: MIDI.

Anwar, A. F., A. Khan, S. K. Saxena, and E. S. M. Mousa. 1996. Interaction between *Meloidogyne incognita* and *Rhizoctonia solani* on soybean. Proceedings of the Second Afro-Asian Nematology Symposium held at Menoufiya, Egypt, 18-22 December 1994:110-113.

Atkinson, G. F. 1892. Some diseases of cotton. Bulletin of Alabama Agricultural Experiment Station 41:61-65.

Atkinson, T. G., J. L. Neal, and J. I. Larson. 1975. Genetic control of the rhizosphere microflora of wheat. Pp. 116-122 in G. W. Bruehl (ed.). Biology and control of soilborne plant pathogens. St. Paul, MN: American Phytopathological Society Pp216.

Ayoub, S. M. 1980. Plant nematology: An agricultural training aid. Sacramento, California: Nemaid Press.

Azad, H. R., J. R. Davis, W. C. Schnathorst, and C. I. Kado. 1985. Relationship between rhizoplane and rhizosphere bacteria and Verticillium wilt resistance in potato. Archives of Microbiology 140:347-351.

Baines, R. C. 1950. Nematodes on citrus. California Agriculture 4:7.

Baines, R. C, S. D. Van Gundy, and E. P. Ducharme. 1978. Nematodes attacking citrus. Pp. 321-345 *in* W. Reuther., E. C. Calavan, and G. E. Carman, eds. The Citrus Industry, Vol. 4. University of California, Davis.

Baker, R. 1968. Mechanisms of biological control of soilborne pathogens. Annual Review of Phytopathology 6:263-294.

Bello, A., A. Navas, and C. Belart. 1986. Nematodes of citrus-groves in the Spanish Levante Ecological study focused to their control. Pp. 217-226 *in* R. Cavalloro., and E. D. Martino, eds. Intergrated Pest Control in Citrus-Groves, Rotterdam, Netherlands.

Bergeson, G. B. 1963. Influence of *Pratylenchus penetrans* alone and in combination with *Verticillium albo-atrum* on growth of peppermint. Phytopathology 53:1164-1166.

Bergeson, G. B. 1972. Concepts of nematode-fungus association in plant disease complexes: A review. Experimental Parasitology 32:301-314.

Bergeson, G. B., S. D. Van Gundy, and I. J. Thomason. 1970. Effect of *Meloidogyne javanica* on rhizosphere microflora and Fusarium wilt of tomato. Phytopathology 60:1245-1249.

Bird, A. F. 1962. The inducement of giant cells by *Meloidogyne javanica* relative to roots. Nematologica 8:1-10.

Bird, G.W. 1981. Management of plant parasitic nematodes in potato production. Pp. 223-243 *in* J. H. Lashomb and R. Casagrande, eds. Advances in potato pest management, Strundsburg, PA: Hutchinson Ross Publisher Company.

Bowen, G. D., and A. D. Rovira. 1976. Microbial colonization of plant roots. Annul Review of Phytopathology 14:121-144.

Bowers, J. H., S. T. Nameth, R. M. Ridel, and R. C. Rowe. 1996. Infection and colonization of potato roots by *Verticillium dahliae* as affected by *Pratylenchus penetrans* and *P. crenatus*. Phytopathology 86:614-621.

Bowman, P., and J. R. Bloom. 1966. Breaking of resistance of tomato varieties to *Fusarium* wilt by *Meloidogyne incognita*. Phytopathology 56:87.

Brisbane, P. G., L. J. Janik, M. E. Tate, and R. F. O. Warren. 1987. Revised structure for the phenazine antibiotic from *Pseudomonas fluorescens* 2-79 (NRRL B-15132). Antimicrobial Agents and Chemotherapy 31:1967-1971.

Broadbent, P., P. K. Barker, N. Franks, and J. Holland. 1977. Effect of *Bacillus* spp. on increased growth of seedlings in steamed and nonsteamed soil. Phytopathology 67:1027-1034.

Brown, M. E. 1981. Microbiology of roots infected with take-all fungus (*Gaeumannomyces graminis* var. *tritici*) in phased sequence of winter wheat. Soil Biology and Biochemistry 13:285-291.

Burr, T. J., M. N. Schroth, and T. Suslow. 1978. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68:1377-1383.

Burrows, P. M. 1987. Interaction concepts for analysis of responses to mixtures of nematode populations. Pp. 82-93 *in* J. A. Veech and D. W. Dickson, eds. Vistas on Nematology, Society of Nematologists, Hyattsville, MD, U.S.A.

Carter, W. W. 1981. The effect of *Meloidogyne incognita* and tissue wounding on severity of seedling disease of cotton caused by *Rhizoctonia solani*. Journal of Nematology 13:374-376.

Cartwright, D. K., and D. M. Benson. 1995. Comparsion of *Pseudomonas* species and applied techniques for biocontrol of Rhizoctonia stem rot of poinsettia. Plant Disease 79:309-313.

Cobb, N. A. 1914. Citrus-root nematode. Journal of Agriculture Research 2:217-230.

Cohn, E. 1964. Penetration of the citrus nematode in relation to root development. Nematologica 10:594-600.

Cohn, E. 1965. On the feeding and histopathology of the citrus nematode. Nematologica 11:594-600.

Cohn, E. 1966. Observation on the survival of free-living stages of the citrus nematode. Nematologica 12:321-327.

Cohn, E. 1976. Report on investigations on nematodes of citrus and subtropical fruit crops in South Africa. Report Citrus Subtropical Fruit Research Institute, Nelspruit, South Africa.

Cohn, E., G. Minz, and S. P. Monselise. 1965. The distribution, ecology and pathogenicity of the citrus nematode in Israel. Israel Journal of Agriculture Research 15:187-200.

Conroy, J. J., R. J. Green, and J. M. Ferris. 1972. Interaction of *Verticillium alboatrum* and the root-lesion nematode *Pratylenchus penetrans* in tomato roots at controlled inoculm densities. Phytopathology 62:362-366.

Cook, R. J., and K. F. Baker. 1983. The nature and practice of biological control of plant pathogens. American Phytopathological Society, St Paul, MN.

Copper, R. 1959. Bacterial fertilizers in the Soviet Union. Soils Fertilizers 22:194-214.

Costa Manso, E. S. B. G and C. S. Huang. 1986. Interacao entre *Meloidogyne incognita* (race 4) eisoladoes de *Rhizocotonia solani*, em *Phaseolus vulgaris*: cv. Rico. Fitopatologia Brasileria 23:857-864.

Davide, R. G. 1971. Survey of the distribution of different plant parasitic nematodes associated with the citrus decline in the Philippines. Laguna, Philippines: University of Philippines, College of Agriculture.

De Freitas, J. R., and J. J. Germida. 1990. A root tissue culture system to study winter wheat-rhizobacteria interactions. Applied Microbiology and Biotechnology 33:589-595.

Dickinson, C. H. 1979. External synergism among organisms inducing disease. Pp. 97-111 *in* J.G. Horsfall and E. B. Cowling, eds. Plant Disease, vol. IV. New York, Academic Press.

Duncan, L. W. 1989. Effect of fenamiphos placement on *Tylenchulus semipenetrans* and yield in Florida citrus orchard. Journal of Nematology 21:703-706.

Duncan, L. W. 1995. Environmental determinants of crop damage by *Tylenchulus semipenetrans* and other nematode parasites of citrus. Pp. 204-212 *in* Proceedings of XXVII International Congress of Tropical Nematology, 4-9 June, Rio Quente, Brasil.

Duncan, L. W., and E. Cohn. 1990. Nematode parasites on citrus. Pp. 321-346 *in* M. Luc., R. A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford, England, CAB Int.

Duncan, L. W., and M. M. El-Morshedy. 1996. Population changes of *Tylenchulus semipenetrans* under localized versus uniform drought in the citrus root zone. Journal of Nematology 28:360-368.

Duncan, L. W., and H. Ferris. 1982. Interactions between phytophagous nematodes mathematical models. Pp. 29-51 *in* D. W. Freckman (ed.). Nematodes in Soil Ecosystems, University of Texas Press.

Duncan, L. W., J. H. Graham, and L. W. Timmer. 1993. Seasonal patterns associated with *Tylenchulus semipenetrans* and *Phytophthora nicotianae* in the citrus rhizosphere. Phytopathology 83:573-581.

Duncan, L. W., J. D. Toole, R. N. Inserra, W. S. Castle, and J. H. O'Bannon. 1994. Comparing two sampling methods to detect *Radopholus citrophilus* in Florida citrus. Proceedings of Soil and Crop Science Society of Florida 53:42-45.

Dunn, D., L. W. Duncan, and J. T. Romeo. 1998. Changes in arginine, PAL activity, and nematode behavior in salinity-stressed citrus. Phytochemistry 49:413-417.

Dunne, C., Y. Moenne-Loccoz, J. McCarthy, P. Higgins, J. Powell, D. N. Dowling, and F. O. Gara. 1998. Combining proteolytic and phloroglucinol-producing bacteria for improved biocontrol of Pythium-mediated damping-off of sugar beet. Plant Pathology-Oxford 47:299-307.

Dunnett, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. Journal of American Statistical Association 50:1096-1121.

Edmunds, J. E., and W. F. Mai. 1966. Effects of *Tricoderma viride*, *Fusarium oxysporum* and fungal enzymes upon the penetration of alfalfa roots by *Pratylenchus penetrans*. Phytopathology 56:1132-1135.

Elad, Y., and I. Chet. 1987. Possible role of competition for nutrients in biocontrol of Pythium damping-off by bacteria. Phytopathology 77:190-195.

El-Borai, K. F., L. W. Duncan, and J. H. Graham. 2000. *Tylenchulus semipenetrans* suppresses infection of citrus fibrous roots by the fungus *Phytophthora nicotianae* (= *parasitica*). Journal of Nematology 32:427-428 (Abstr.).

El-Borai Kora, F. E., L. W. Duncan, and J. H. Graham. 2001. Mechanisms by which *Tylenchulus semipenetrans* may mitigate virluence of *Phytophthora nicotianae* to citrus seedlings. Phytopathology 91: S137

El-Sherif, A. C., and M. A. Elwakil. 1991. Interaction between *Meloidogyne incognita* and *Agrobacterium tumifaciens* or *Fusarum oxysporum* f. sp. *lycopersici* on tomato. Journal of Nematology 23:239-242.

Evans, K., and P. P. J. Haydock. 1993. Interaction of nematodes with root-rot fungi. Pp. 104-133 *in* M.W. Khan, ed. Nematode Interactions, New York: Chapman and Hall.

Faulkner, L. R., and W. J. Bolander. 1969. Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint; Effects of soil temperature. Phytopathology 59:868-870.

Faulkner, L. R., W. J. Bolander, and C. B. Skotland. 1970. Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint; Influence of the nematode as determined by a double root technique. Phytopathology 60:100-103.

Ferraz, S., and B. Lear. 1976. Observation on the growth and maintenance of *Rotylenchulus robustus*, *Criconemoides curvatus* and *Paratylenchus dianthus* (Nematode Pests of *Dianthus caryophyllus*). Experimentiae 22:272-277.

Fisher, J. 1993. CIBA-Geigy addresses *Phytophthora* resistance to Ridomil. Citrus Industry 74:28-29.

Florida Agriculture Statistics Service. 2000. Citrus, commercial citrus inventory, preliminary report. Florida Department of Agriculture and Consumer services.

France, L. J., and T. A. Wheeler. 1993. Interaction of plant parasitic nematodes with wilt inducing fungi. Pp. 80-103 *in* M.V. Khan, ed. Nematode Interactions. New York: Chapman and Hall.

Giesler, L. J., and G. Y. Yuen. 1998. Evaluation of *Stenotrophomonas maltophilia* strain C3 for biocontrol of brown patch disease. Crop Protection 17:509-513.

Golden, J. K., and S. D. Van Gundy. 1972. Influence of *Meloidogyne incognita* on root knot development by *Rhizoctonia solani* and *Thielaviopsis basicola* in tomato. Journal of Nematology 4:225.

Golden, J. K., and S. D. Van Gundy. 1975. A disease complex of okra and tomato involving the nematode, *Meloidogyne incognita*, and the soil inhabiting fungus, *Rhizoctonia solani*. Phytopathology 65:265-272.

Graham, J. H. 1990. Evaluation of tolerance of citrus rootstocks to *Phytophthora* root rot in chlamydospore-infested soil. Plant Disease 74:743-746.

Graham, J. H. 1995. Root regeneration and tolerance of citrus rootstocks to root rot caused by *Phytophthora nicotianae*. Phytopathology 85:111-117.

Graham, J. H., and L. W. Duncan. 1997. Suppression of *Phytophthora nicotianae* in citrus roots by the citrus nematode (*Tylenchulus semipenetrans*). Phytopathology (Suppl.) 87 S35.

Graham, J. H., and J. A. Menge. 1999. Root diseases. Pp. 126-135 *in* L. W. Timmer., and L. W. Duncan, eds. Citrus Health Management. APS press, St. Paul, MN.

Graham, J. H., and L. W. Timmer. 1992. *Phytophthora* diseases of citrus. Pp. 250-269 *in* J. Kumar., H. S. Chaube, U. S. Singh, and A. N. Mukhopadhyay, eds. Plant Diseases of International Importance. Vol. 3. Diseases of Fruit Crops. New Jersey: Prentice-Hall.

Gray, F. A., G. D. Griffin, D. A. Johnson, J. W. Eckert, and J. E. Kazimir. 1990. Interrelationships between *Meloidogyne hapla* and *Phytophthora megasperma* f. sp. *Medicaginis* in seedlings damping-off and root infection of alfalfa. Phytopathology 80:228-232.

Griffin, G. D., and F. A. Gray. 1994. Pathological relationship of *Meloidogyne hapla* and *Phytophthora megasperma* f. sp. *medicaginis* in twelve *Medicago sativa* L cultivars. Nematropica 24:35-43.

Griffin, G. D., F. A. Gray, D. A. Johnson and D. L. Crebs. 1993. Pathological relationship of *Meloidogyne hapla* and *Phytophthora megasperma* f. sp. *medicaginis* in *Medicago sativa* L.: importance of inoculation timing, soil texture, and temperature. Nematropica 23:183-193.

Griffin, G. D., and B. D. Thyr. 1988. Interactions of *Meloidogyne hapla* and *Fusarium oxysporum* f. sp. *medicaginis* on alfalfa. Phytopathology 78:421-425.

Gutierrez, R. O. 1947. The nematode of citrus roots, *Tylenchulus semipenetrans*, in Argentina. Review Interest of Agriculture 1:119-146.

Hall, G. 1993. An integrated approach to the analysis of variation in *Phytophthora nicotianae* and a redescription of the species. Mycological Research 97:559-574.

Harrison, A. L., and P. A. Young. 1941. Effect of root-knot nematode on tomato wilt. Phytopathology 31:749-752.

Hasan, A. 1984. Synergistic between *Heterodera cajani* and *Fusarium udum* attacking *Cajanus cajan*. Nematologia Mediterranea 12:159-162.

Hasan, A. 1985. Breaking resistance in chilli to root-knot nematode by fungal pathogens. Nematologica 31:210-217.

Hasan, A. 1988. Interaction between *Pratylenchus coffeae* and *Pythium aphaniderum* and /or *Rhizocotonia solani* on chrysanthemum. Journal of Phytopathology 123:227-232.

Heald, C. M., and J. H. O'Bannon. 1987. Citrus decline caused by nematodes.V. Slow decline. Nematology Circulation Number 143. Florida Department of Citrus, Lake Alfred, FL.

Heller, J., J. Shalhevet, and A. Goel. 1973. Response of a citrus orchard to soil water and soil salinity. Pp. 409-419 *in* A. Goel, ed. Physiological Aspects of Soil Water and Salt in Ecosystems. Ecological Studies, Vol. 4. New York: Springer-Verlag.

Hendrickx, G. J., W. A. Coolen, and R. Moermans. 1976. Influence de la nature et de la densite des liquides de separation sur les nematodes penedant le processus de centrifugation-flotation. Nematologia Mediterranea 4:31-40.

Hillocks, R. J. 1986. Localized and systemic effects of root-knot nematode on incidence and severity of *Fusarium* wilt in cotton. Nematologica 32:202-208.

Holdeman, Q. L., and T. W. Graham. 1954. Effect of sting nematode on expression of *Fusarium* wilt in cotton. Phytopathology 44:683-685.

Howie, W. J. 1985. Factors affecting colonization of wheat roots and suppression of take-all by Pseudomonads antagonistic to *Gaeumannomyces graminis* var. *tritici*. Ph.D. Dissertation. Pullman, Washington State University.

Howie, W. J., R. J. Cook, and D. M. Weller. 1987. Effects of soil matric potential and cell motility on wheat root colonization by fluorescent pseudomonads suppressive to take-all. Phytopathology 77:286-292.

Howie, W. J., and E. Echandi. 1983. Rhizobacteria influence of cultivar and soil type on plant growth and yield of potato. Soil Biology and Biochemistry 15:127-132.

Howie, W. J., and T. V. Suslow. 1991. Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in the cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. Molecular Plant-Microbe Interaction 4:393-399.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Report 57:1025-1028.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Report 48:492.

Jonathan, E. L., and G. Rajendran. 1998. Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cubense* on banana. Nematologia Mediterranea 26:9-11.

Jordaan, E. M., G. C. Loots, W. I. Jooste, and D. de Waele. 1987. Effects of root lesion nematodes (*Pratylenchus brachyurus* Godfrey and *P. zeae* Graham) and *Fusarium moniliforme* Sheldon alone or in combination, on maize. Nematologica 33:213-219.

Jorgenson, E. C. 1970. Antagonistic interaction between *Heterodera schachtii* Schmit and *Fusarium oxysporum* (Woll).on sugarbeets. Journal of Nematology 2:393-398.

Kawamoto, S. O., and J. W. Lorbeer. 1976. Protection of onion seedlings from *Fusarium oxysporum* f. sp. *cepae* by seed and soil infestation with *Pseudomonas cepacia*. Plant Disease Report 60:180-191.

Kerr, A. 1956. Some interactions between plant roots and pathogenic soil fungi. Australian Journal of Biological Science 9:45-52.

Khan, M. R., and M. Akram. 2000. Effects of certain antagonistic fungi and rhizobacteria on wilt disease complex of tomato caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici*. Nematologica Mediterranea 28:139-144.

Khan, M. W., and J. Muller. 1982. Interaction between *Rhizoctonia solani* and *Meloidogyne hapla* on radish in gnotobiotic culture. Libyan Journal of Agriculture 11:113-40.

Khan, M. W., and S. A. Nejad-Hosseini. 1991. Interaction of *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *ciceris* on some chickpea cultivars. Nematologia Mediterranea 119:161-163.

Khew, K. L., and G. A. Zentmeyer. 1973. Chemotactic response of zoospores of five species of *Phytophthora*. Phytopathology 63:1511-1517.

King, E. B., and J. L. Parke. 1993. Biological of Aphanomyces root rot and Pythium damping off by *Pseudomonas cepacia* AMMD on four pea cultivars. Plant Disease 77:1185-1185.

Klinkowski, M. 1970. Catastrophic plant diseases. Annual Review of Phytopathology 8:37-60.

Kloepper, J. W. 1993. Plant growth-promoting rhizobacteria as biological control agents. Pp 255-274 *in* F. B., Jr. Metting, ed. Soil microbial ecology. NewYork: Marcel Dekker.

Labuschagne, N., F. A. Van Der Vegte, and J. M. Kotze. 1989. Interaction between *Fusarium solani* and *Tylenchulus semipenetrans* on citrus roots. Phytophylactica 21:29-33.

Lambert, B., F. Leyns, L. Van Rooyen, F. Gossele, Y. Papon, and J. Swings. 1987. Rhizobacteria of maize and their antifungal activities. Applied and Environmental Microbiology 53:1866-1871.

LaMondia, J. A. 1999. Effects of *Pratylenchus penetrans* and *Rhizoctonia fragariae* on vigor and yield of strawberry. Journal of Nematology 31:418-423.

Larkin, R. P., and D. R. Fravel. 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of fusarium wilt of tomato. Plant Disease 82:1022-1028.

Liu, Z. L., and J. B. Sinclair. 1993. Colonization of soybean roots by *Bacillus megaterium* B153-2-2. Soil Biology and Biochemistry 25:849-855.

Lutz, A. L., and J. A. Menge. 1986. Citrus root health II. *Phytophthora* root rot. Citrograph 72:33-39.

MacDonald, J. D., and J. M. Duniway. 1978. Influence of the matric and osmotic components of water potential on zoospore discharge in *Phytophthora*. Phytopathology 68:751-757.

MacGuidwin, A. E., and D. I. Rouse. 1990. Role of *Pratylenchus penetrans* in the potato early dying disease of russet Burbank Potato. Phytopathology 80:1077-1082.

Machmer, J. H. 1958. Effect of soil salinity in nematodes in citrus and papaya plantings. Journal of Rio Grande Valley Horticulture Society 12:57-60.

Maggenti, A. R. 1962. Production of gelatinous matrix and taxonomic signification in *Tylenchulus* (Nematoda-Tylenchulinae). Proceedings of Washington Helminthological Society 29:139.

Mai, W. F., and G. S. Abawi. 1987. Interactions among root-knot nematodes and Fusarium wilt fungi on host plants. Annual Review of Phytopathology 25:317-338.

Malajczuk, N. 1983. Microbial antagonism to *Phytophthora*. Pp. 197-218 *in* D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. Phytophthora: Its Biology, Taxonomy, Ecology and Pathology. St. Paul, MN, APS Press.

Maloney, P. E., A. H. C. Van Bruggen, and S. Hu. 1997. Bacterial community structure in relation to the carbon environment in lettuce and tomato rhizospheres and in bulk soil. Microbial Ecology 34:109-117.

Mao, W., J. A. Lewis, K. P. Hebbar, and R. D. Lumsden. 1997. Seed treatment with a fungal or a bacterial antagonist for reducing corn damping-off caused by species of *Pythium* and *Fusarium*. Plant Disease 81:450-454.

Mao, W., J. A. Lewis, R. D. Lumsden, and K. P. Hebbar. 1998a. Biocontrol of selected soilborne diseases of tomato and pepper plants. Crop Protection 17:535-542.

Mao W., R. D. Lumsden, J. A Lewis, and K. P. Hebbar. 1998b. Seed treatment using pre-infiltration and biocontrol agents to reduce damping-off of corn caused by species of *Pythium* and *Fusarium*. Plant Disease 82:294-299.

Martin, J. P., and S. D. Van Gundy. 1963. Influence of soil phosphorus level on the growth of sweet orange seedlings and the activity of the citrus nematode *Tylenchulus semipenetrans*. Soil Science 96:128-125.

Mashela, P., L. W. Duncan, and R. McSorley. 1992. Salinity reduces resistance to *Tylenchulus semipenetrans* in citrus rootstocks. Nematropica 22:7-12.

McClure, M. A., T. H. Kruk, and I. Misaghi. 1973. A method for obtaining quantities of clean *Meloidogyne* eggs (Vegetables, Nematodes). Journal of Nematology 4:230.

McLean, K. S., and G. W. Lawrence. 1993a. Interrelationship of *Heterodera glycines* and *Fusarium solani* in sudden death syndrome. Journal of Nematology 25:434-439.

McLean, K. S., and G. W. Lawrence. 1993b. Localized influence of *Heterodera glycines* on sudden death syndrome of soybean. Journal of Nematology 25:674-678.

McLean, K. S., and G. W. Lawrence. 1995. Development of *Heterodera glycines* as affected by *Fusarium solani*, the causal agent of sudden death syndrome of soybean. Journal of Nematology 27:70-77.

Meyer, S. L. F., D. P. Roberts, D. J. Chitwood, L. K. Carta, R. D. Lumsden, and W. Mao. 2001. Application of *Burkholderia cepacia* and *Tricoderma virens*, alone and in combinations, against *Meloidogyne incognita* on bell pepper. Nematropica 31:75-86.

Millus, E. A., and C. S. Rothrock. 1997. Efficacy of bacterial seed treatments for controlling *Pythium* root rot of winter wheat. Plant Disease 81:180-184.

Mircetich, S. M., and G. A. Zentmeyer. 1970. Germination of chlamydospores of *Phytophthora*. Pp. 112-115 *in* T. A. Tousson, R. V. Bega, and P. E. Nelson, eds. Root Diseases and Soilborne Pathogens. Los Angles, University of California Press.

Mishustin, E. N. 1963. Bacterial fertilizers and their effectiveness. Microbiology 32:774-778.

Mitchell, D. J., and M. E. Kannwischer-Mitchell. 1992. *Phytophthora.* Pp 31-38 *in* L. L. Singleton, J. D. Michial and C. M. Rush, eds. Methods for research on soilborne phytopathogenic fungi. APS press, St. Paul, Minnesota.

Mitchell, D. J., and G. A. Zentmeyer. 1971. Effects of oxygen and carbon dioxide tensions on sporangium and oospore formation by *Phytophthora* spp. Phytopatholoy 61:807-812.

Mitchell, R., and M. Alexander. 1961. The mycolytic phenomenon and biological control of *Fusarium* in soil. Nature 190:109-110.

Mitchell., R, and E. Hurwitz. 1964. Suppression of *Pythium debaryanum* by lytic rhizosphere bacteria. Phytopathology 55:156-158.

Mitchell, R., and E. Hurwitz. 1965. Suppression of *Pythium debaryanum* by lytic rhizosphere bacteria. Phytopathology 55:156-158.

Moorman, G. W., J. S. Huang, and N. T. Powell. 1980. Localized influence of *Meloidogyne incognita* on Fusarium wilt resistance of flue-cured tobacco. Phytopathology 70:969-970.

Morris, P. E., and E. W. B. Ward. 1992. Chemoattraction of zoospores of the soybean pathogen, *Phytophthora sojae*, by isoflavones. Physiology and Molecular Plant Pathology 40:17-22.

Mouza, B. E., and J. M. Webster. 1982. Suppression of alfalfa growth by concomitant populations of *Pratylenchus penetrans* and two *Fusarium* species. Journal of Nematology 14:364-367.

Murashige, T., and D. P. H. Tucker. 1969. Growth factor requirements of citrus tissue culture. Pp. 1151-1161 *in* H. D. Chapman, ed. Proceedings of the first international citrus symposium, vol. 3., Riverside California.

Negron, J. A., and N. Acosta. 1989. The *Fusarium oxysporum* f. sp. *coffeae-Meloidogyne incognita* complex in "Bourbon" coffee. Nematropica 19:161-168.

Nicholson, A. J. 1933. The balance of animal populations. Journal of Animal Ecology 2:132-178.

Neipp, P. W., and J. O. Becker. 1999. Evaluation of biocontrol activity of rhizobacteria from *Beta vulgaris* against *Heterodera schachtii*. Journal of Nematology 31:54-61.

Noguera, R., and B. G. Smits. 1982. Variaciones en la microflora de la rhizosfera del tomate infectado con *Meloidogyne incognita*. Agronomica Tropical 32:147-154.

Norton, D. C. 1978. Ecology of plant parasitic nematodes. New York: Wiley.

O'Bannon, J. H. 1968. The influence of an organic soil amendment on infectivity and reproduction of *Tylenchulus semipenetrans*. Phytopathology 58:597-601.

O'Bannon, J. H., C. R. Leathers, and H. W. Reynolds. 1967. Interactions of *Tylenchulus semipenetrans* and Fusarium species on rough lemon. Phytopathlogy 57:414-417.

O'Bannon, J. H., J. D. Radewald, and A. T. Tomerlin. 1972. Population fluctuation of three parasitic nematodes in Florida citrus. Journal of Nematology 4:194-199.

O'Bannon, J. H., H. W. Reynolds, and C. R. Leathers. 1966. Effects of temperature, development and reproduction of *Tylenchulus semipenetrans*. Nematologica 12:483-487.

Orion, D., and G. Kritzman. 1991. Antimicrobial activity of *Meloidogyne javanica* gelatinous matrix. Revue de Nematologie 14:481-484.

Osburn, R. M., M. N. Schroth, J. G. Hancock, and M. Hendson. 1989. Dynamics of sugar beet seed colonization by *Pythium ultimum* and *Pseudomonas* species: Effects on seed rot and damping-off. Phytopathology 79:709-716.

Papert, A., and C. J. Kok. 1999. Microbiology of egg masses of the root knot nematode *Meloidogyne fallax*. International Congress of Phytopathology, Edinburgh, Switzerland.

Patel, D. B., Patel, D. J, and N. B. patel. 2000. Interaction of reniform nematode, *Rotylenchulus reniformis* and wilt, *Fusarium oxysporum* f. sp. *ricini* in castor (*Ricinus communis* L.). Annals of Plant Protection Science 8:209-211.

Pitcher, R. S. 1978. Interactions of nematodes with other pathogens. Pp. 63-77 *in* J. F. Southey ed. London: Her Majesty's Stationary Office.

Powell, N. T. 1971a. Interaction between nematode and fungi in disease complexes. Annual Review of Phytopathology 9:253-274.

Powell, N. T. 1971b. Interaction of plant parasitic nematodes with other disease causing agents. Pp. 119-136 *in* B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Plant parasitic nematodes, vol. 2. New York: Academic Press.

Powell, N. T. 1979. Internal synergism among organisms inducing disease. Pp. 113-133 *in* J. G. Horsfall, and E. B. Cowling, eds. Plant disease, an advanced treatise. vol. 4, New York: Academic Press.

Powell, N. T., P. L. Melendez, and C. K. Batten. 1971. Disease complexes in Tobacco involving *Meloidogyne incognita* and certain soil-borne fungi. Phytopathology 61:1332-1337.

Powelson, M. L., and R. C. Rowe. 1993. Biology and management of early dying of potato. Annual Review of Phytopathology 31:111-126.

Prot, J. 1993. Biochemical and genetic basis of fungus-nematode interactions. Pp. 273-285 *in* M. V. Khan, ed. Nematode Interactions. New York: Chapman and Hall.

Rai, P. K., and K. P. Singh. 1996. Pathogenicity and histopathology of *Heterodera cajani* and two isolates of *Fusarium udum* on pigeonpea. National Academy of Science Letters 19:1-2, 4-7.

Reynolds, H. W., and J. H. O'Bannon. 1963. Decline of grapefruit in relation to citrus nematode populations and tree recovery after chemical treatment. Phytopathology 53:1011-1015.

Reynolds, H. W., J. H. O'Bannon, A. T. Tomerlin, E. L. Nigh, and D. R. Rodney. 1970. The influence of various ecological factors on the survival of *Tylenchulus semipenetrans*. Proceedings of Soil and Crop Science Society of Florida 30:336-370.

Ribeiro, O. K. 1983. Physiology of asexual spourlation and spore germination in *Phytophthora*. Pp. 55-70 *in* D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. *Phytophthora*: Its biology, taxonomy, ecology and pathology, St. Paul, MN: APS Press.

Rovira, A. D., and G. B. Wildermuth. 1981. The nature and mechanisms of suppression. Pp. 17:385-415 *in* M. J. C. Asher, and J. P. Shipton, eds. Biology and Control of Take-all. Londen/New York: Academic Press.

Rowe, R. C., J. R. Davis, M. L. Powelson, and D. I. Rouse. 1987. Potato early dying: Causal agents and management strategies. Plant Disease 71: 482-489.

Roy, K. W., G. W. Lawrence, H. H. Hodges, K. S. Mclean, and J. F. Killebrew. 1989. Sudden death syndrome of soybean: *Fusarium solani* as incitant and relation of *Heterodera glycines* to disease severity. Phytopathology 79:191-197.

Sakhuja, P. K., and C. L. Sethi. 1986. Multiplication of *Meloidogyne javanica* as affected by *Fusarium solani* and *Rhizoctonia bataticola* on groundnut. Indian Journal of Nematology 16:1-3.

Sandler, H. A., L.W. Timmer, J. H. Graham, and S. E. Zitko. 1989. Effect of fungicide applications on populations of *Phytophthora parasitica* and on feeder root densities and fruit yield of citrus trees. Plant Disease 73:902-906.

Sankaralingam, A., and E. C. McGawley. 1994. Interrelationships of *Rotylenchulus reniformis* with *Rhizoctonia solani* on cotton. Journal of Nematology 26:475-485.

Santo, G. S., and O. V. Holtzmann, 1970. Interrelationships of *Pratylenchus zeae* and *Pythium graminicola* on sugarcane. Phytopathology 60:1537(Abstr.).

Sasser, M. 1990. Identification of bacteria through fatty acid analysis. Pp. 199-204 *in* Z. Klement., K. Rudolph, and D. C. Sands, eds. Methods in Phytobacteriology. Akademiai Kiado, Budapest.

Schneider, H., and R. C. Baines. 1964. *Tylenchulus semipenetrans* parasitism and injury to orange tree roots. Phytopathology 54:1202:1206.

Schroth, M. N., and J. G. Hancock. 1981. Selected topics in biological control. Annual Review of Microbiology 35:453-476.

Schroth, M. N., and J. G. Hancock. 1982. Disease-suppressive soil and root colonizing bacteria. Science 216:1376-1381.

Seinhorst, J. W. 1965. The relationship between nematode density and damage to plants. Nematologica 11:137-154.

Shen, C., L. Bower, D. C. Erwin, and P. H. Tsao. 1983. Formation of sex organs in the A1 mating type of *Phytophthora infestans* induced chemically by A2 isolates of other species of *Phytophthora*. Canadian Journal of Botany 61:1462-1466.

Sidhu, G. S., and J. M. Webster. 1977. Predisposition of tomato to the wilt fungus (*Fusarium oxysporum lycopersici*) by the root-knot nematode (*Meloidogyne incognita*). Nematologica 23:436-442.

Sidhu, G. S., and J. M. Webster. 1981. Genetics of plant nematode interaction. Pp. 61-87 *in* Zuckerman and R. A. Rohde, eds. Plant parasitic nematodes, Vol.III, New York: Academic Press.

Singh, D. B., P. P. Reddy, and S. R. Sharma. 1981. Effect of root-knot nematode, *Meloidogyne incognita* on Fusarium wilt of french beans. Indian Journal of Nematology 11:84-85.

Sneh, B. 1981. Use of rhizosphere chitinolytic bacteria for biological control of *Fusarium oxysporum* f. sp. *dianthi* in carnation. Phytopathological Zoology 100:251-256.

Sommers, L. E., R. F. Harris, N. F. Dalton., and W. R. Gardner. 1970. Water potential relations of three root-infecting Phytophthora species. Phytopathology 60:932-934.

Starr, J. L., M. Y. Shim, T. A. Lee Jr, and C. E. Simpson. 1996. Additive effects of *Meloidogyne arenaria* and *Sclerotium rolfsii* on peanut. Journal of Nematology 28:99-106.

Stolzy, L. H., S. D. Van Gundy, C. K. Labanauskas, and T. E. Szuskiewicz. 1963. Response of *Tylenchulus semipenetrans* infected citrus seedlings to soil aeration and temperature. Soil Science 96:292-298.

Suslow, T. V. 1982. Role of root colonizing bacteria in plant growth. Pp. 187-223 *in* M. S. Mount and G. S. Lacy, eds. Phytopathogenic prokaryotes, vol. 1. New York: Academic Press.

Suslow, T. V., and M. N. Schroth. 1982. Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathology 72:111-115.

Taylor, C. E. 1979. *Meloidogyne* interrelationships with microorganisms. Pp. 375-398 *in* F. Lamberti., and C. E. Taylor, eds. Root knot nematodes (*Meloidogyne* Species) systematics, biology and control, London: Academic Press.

Taylor, C. E. 1990. Nematode interaction with other pathogens. Annuals of Applied Biology 116:405-416.

Thomas, E. E. 1913. A preliminary report of nematode observed on citrus roots and its possible relation with the mottled appearance of citrus trees. Circulation of California Agriculture Experiment Station No. 85.

Thomas, E. E. 1923. The citrus nematode, *Tylenchulus semipenetrans*. California Agriculture Experiment Station Tech. paper 2:35.

Thomashow, L. S., and D. M. Weller. 1988. Role of phenazine antibiotic in suppression of *Gaeumannomyces graminis* var. *tritici*. Journal of Bacteriology 170:3499-3508.

Thorne, G. 1961. Principles of Nematology. New York: McGraw-Hill.

Timmer, L. W., J. H. Graham, H. A. Sandler, and S. E. Zitko. 1988. Populations of *Phytophthora parasitica* in citrus orchards and tree response to fungicide applications. Citrus Industry 69:40-44.

Timmer, L. W., and J. A. Menge. 1988. *Phytophthora*-induced diseases. Pp. 22-24 *in* J. O. Whiteside., S. M. Garnsey, and L. W. Timmer, eds. Compendium of citrus diseases, St. Paul, MN, APS Press.

Timmer, L. W., H. A. Sandler, J. H. Graham, and S. E. Zitko. 1989. *Phytophthora* feeder root rot of bearing citrus: Fungicide effects on soil populations of *Phytophthora parasitica* and citrus tree productivity. Proceeding of of the Annual Meeting of the Florida State Horticultural Society 102:5-9.

Tsao, P. H. 1969. Studies on the saprophytic behavior of *Phytophthora parasitica* in soil. Pp. 1221-1230 *in* H. Chapman ed. Proceeding the First International Citrus Symposium, vol. III. Riverside, CA.

Tsao, P. H. 1971. Clamydospore formation in sporangium-free liquid cultures of *Phytophthora parasitica*. Phytopathology 61:1412-1413.

Turney, J. K., J. K. Menge, C. H. Yang, and D. A. Cooksey. 1992. Biocontrol of citrus root rot using soilborne bacteria. Phytopathology 82:1080 (Abstr.).

Valle-Lamboy, S., and A. Ayala. 1980. Pathogenicity of *Meloidogyne incognita* and *Pratylenchus zeae*, and their association with *Pythium graminicola* on roots of sugarcane in Puerto Rico. University of Puerto Rico: Journal of Agriculture 64:338-347.

Van Gundy, S. D. 1958. The life history of the citrus nematode *Tylenchulus semipenetrans* Cobb. Nematologica 3:283-294.

Van Gundy, S. D. 1984. Nematodes. Pp. 129-131.*in* M. Klein (ed.), Intergated pest managemet for citrus, Publ. 3303. University of California, Davis.

Van Gundy, S. D, J. D. Kirkapatrick, and J. Golden. 1977. Nature and role of metabolic leakage from root-knot nematode galls and infection by *Rhizocotonia solani*. Journal of Nematology 9:113-121.

Van Gundy, S. D., and J. D. Kirkapatrick. 1964. Nature of resistance in certain citrus roots to citrus nematode. Phytopathology 54:419-427.

Van Gundy, S. D., and J. P. Martin. 1961. Influence of *Tylenchulus semipenetrans* on the growth and chemical composition of sweet orange seedlings in soils of various exchangeable cation ratios. Phytopatholgy 51:146-151.

Van Gundy, S. D., J. P. Martin, and P. H. Tsao. 1964. Some soil factors influencing reproduction of the citrus nematode and growth reduction of sweet orange seedlings. Phytopathology 54:294-299.

Van Gundy, S. D., and J. W. Meagher. 1977. Citrus nematode (*Tylenchulus semipenetrans)* problems worldwide. Proceeding of the International Society of Citruculture 3:823-826.

Van Gundy, S. D., and P. H. Tsao. 1963. Infecting citrus seedlings with the citrus nematode, *Tylenchulus semipenetrans*. Phytopathology 53:228-229.

Van Peer, R., G. I. Niemann, and B. Schippers. 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81:728-734.

Vojinovic, Z. D. 1973. The influence of microorganisms following *Ophiobolus graminis* Sacc. on its further pathogenicity. European Mediterranean Plant Protection Organization Bulletin 9:91-101.

Walker, N. A., T. L. Kirkpatrick, and C. S. Rothrock. 1999. Effect of temperature on and histopathology of the interaction between *Meloidogyne incognita* and *Thielaviopsis basicola* on cotton. Phytopathology 89:613-617.

Wallace, H. R. 1983. Interactions between nematodes and other factors on plants. Journal of Nematology 15:221-227.

Wallace, H. R. 1989. Environment and plant health; A nematological perception. Annual Review of Phytopathology 27:59-75.

Walter, I. C. 1965. Host resistance as it relates to root pathogens and soil microorganisms. Pp. 314-320 *in* K. F. Barker and W. C. Snyder, eds. Ecology of soilborne plant pathogens, Berkeley: University of California Press.

Wang, E. L. H, and G. B. Bergeson. 1974. Biochemical changes in root exudates and xylem sap of tomato plants infected with *Meloidogyne incognita*. Journal of Nematology 6:194-202.

Webster, J. M. 1985. Interaction of *Meloidogyne* with fungi on crop plants. Pp. 183-192 *in* J. N. Sasser, and C. C. Carter, eds. An advance treatise on *Meloidogyne*, vol. 1. Raleigh: North Carolina State University Graphics.

Wei, G., J. W. Kloepper, and S. Tuzun. 1991. Induction of systemic resistance of cucumber to *Colletotrichum orbiculare*, by select strains of plant growth-promoting rhizobacteria. Phytopathology 81:1508-1512.

Weinhold, A. R., R. L. Dodman, and T. Bowman. 1972. Influence of exogenous nutrition on virulence of *Rhizoctonia solani*. Phytopathology 62:278-281.

Weller, D. M. 1983. Colonization of wheat roots by a fluorescent pseudomonad suppressive to take all. Phytopathology 73:1548-1553.

Weller, D. M. 1985. Application of fluorescent pseudomonads to control root diseases. Pp. 137-140 *in* C. S. Parker, A. D. Rovira, K. J. Moore, P. T. W. Wong, and J. F. Kollmorgan, eds. Ecology and management of soilborne plant pathogens, St. Paul; MN: American Phytopathological Society.

Weller, D. M. 1986. Effects of wheat genotype on root colonization by a take-all suppressive strain of *Psudomonas fluorescens*. Phytopathology 76:1059 (Abstr.).

Weller, D. M. 1988. Biological control of soilborne pathogens in the rhizopshere with bacteria. Annual Review of Phytopathology 26:379-407.

Westcott, S. W., and D. A. Kluepfel. 1993. Inhibition of *Criconemella xenoplax* egg hatch by *Pseudomonas aureofaciens*. Phytopathology 83:1245-1249.

Westerlund, F. U., Jr., R. N. Campbell, and K. A. Simble. 1974. Fungal root rot and wilt of chick-pea in California. Phytopathology 64:432-436.

Wheeler, T. A., and R. M. Riedel. 1994. Interaction among *Pratylenchus penetrans*, *Pratylenchus scribneri*, and *Verticillium dahliae* in the potato early dying disease complex. Journal of Nematology 26:228-234.

Whiteside, J. O., S. M. Garnsey, and L. W. Timmer. 1988. Compendium of citrus diseases. Pp 80 APS Press, St. Paul, MN.

Whitney, E. D. 1974. Synergistic effect of *Pythium ultimum* and the additive effect of *P. aphanidermatum* with *Heterodera schachtii* on sugarbeet. Phytopathology 64:380-383.

Yang, H., N. T. Powell, and K. R. Barker. 1976. Interaction of concomitant species of nematodes and *Fusarium oxysporum* f. sp *vasinvectum* on cotton. Journal of Nematology 8:74-80.

Zadoks, J. C., and R. D. Schein. 1979. Epidemiology and plant disease management. Oxford: Oxford University Press.

Zentmyer, G. A. 1961. Chemotaxis of zoospores for root exudates. Science 133:1595 1596.

Zheng, X. Y., and J. B. Sinclair. 1996. Chemotactic response of *Bacillus megaterium* strain B153-2-2 to soybean root and seed exudates. Physiological and Molecular Plant Pathology 48:21-35.

Zitko, S. E., L.W. Timmer, and W. S. Castle. 1987. Survey of Florida citrus nurseries for *Phytophthora* spp. Proceeding of the Annual meeting of the Florida State Horticultural Society 100:82-85.

BIOGRAPHICAL SKETCH

Fahiem E. El-Borai was born in El-Mansoura, Dakahleia governerate, Egypt on 6 January, 1966. He received a B.S. in plant protection in 1987. Fahiem was appointed to a position as an assistant professor in the Plant Protection Department at El-Zagazig University in Egypt in 1988. He began working toward his Master of Science degree in the area of plant-parasitic nematodes. Fahiem conducted research and taught agricultural zoology courses (morphology, taxonomy and physiology) for undergraduate students at the Faculty of Agriculture, El-Zagazig University.

Fahiem obtained his Master of Science degree in agricultural zoology (nematology) in 1993. He continued teaching at the same university until 1996. He was awarded a scholarship from the Egyptian government to pursue his Ph.D. studies abroad. In the summer of 1997, he enrolled in the graduate program (nematology) of the Entomology and Nematology Department at the University of Florida under Dr. Larry W. Duncan's supervision. He considers himself very fortunate to be enrolled in one of the greatest graduate programs in nematology, with excellent scientists who are a pleasure to work with. He completed his research at the Citrus Research and Education Center in Lake Alfred, Florida.

After completing his Ph.D. program, Fahiem plans to work as a postdoctoral researcher to gain more experience in his subject area. Later, he will return to Egypt to fulfill an appointed position as an associate professor at El-Zagazig University. Fahiem will teach and conduct research and he hopes to deliver the excitement and enthusiasm to his students in Egypt that he has experienced from his professors in the U.S.A.

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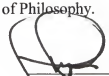

Barry W. Duncan, Chair
Professor of Entomology and Nematology

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James H. Graham
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald W. Dickson
Professor of Entomology and Nematology

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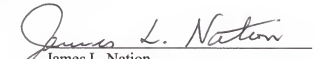

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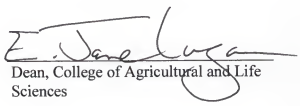
This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 2001



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